A microfluidic model for *in vitro* studies of thromboembolism and thrombolysis

by

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Abstract

Thromboembolism originates from obstruction of blood vessels with pathological emboli. It is a primary reason for ischemic strokes, pulmonary embolism and myocardial infarction. The correlation between occlusive clot structure, mechanical properties and its resistance to lysis remains unexplored. This thesis describes the development of a microfluidic platform for *in vitro* studies of thromboembolism and thrombolysis. A “double-modeling” approach was developed to investigate the motion of an artificial embolus (a hydrogel microparticle) through an artificial blood vessel (a constriction-based microchannel with a circular cross-section).

A pressure-driven microfluidic platform was established using agarose hydrogel particles (microgels). We explored the entrance of a microgel into a constriction with a tapered region and water loss by the confined microgel. By studying the behavior of agarose microgels with various dimensions and mechanical properties in narrow microchannels with varying geometries, we established universal principles governing the flow of microgels under spatial constraints. The observed principles agreed excellently with a theoretical model developed for bi-axially deformed microgel. We also showed a non-monotonic change in the flow rate of liquid through the occlusive microgel, which was governed by its progressive confinement in the tapered region of the channel.

The developed platform was extended to fibrin microgels, in order to delineate the relationship between the composition, dimensions, and mechanical properties of fibrin hydrogels with respect to their occlusive
behavior, relaxation after release from confinement and their ability to lyse. For the same strain experienced in the constriction, soft fibrin microgels with thicker fibers exhibited stronger irreversible deformation, compared to microgels with a denser network structure. Moreover, the former gels are more prone to liquid permeation and fibrinolysis.

Lastly, we fabricated bifurcating microchannels with a nearly circular cross-section over multiple generations of sizes. Occlusive fibrin microgels caused redistribution of liquid within the network, thus mimicking blood-perfusion abnormalities in pulmonary embolism.
Acknowledgments

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Preface

The thesis is based on key projects which have been either published, submitted, or in preparation for peer-reviewed scientific journals. All manuscripts were written by Yang Li with critical comments and revisions by Eugenia Kumacheva and Arun Ramachandran and corresponding collaborators. The contributions of Yang Li and other co-authors are provided in detail below.

Chapter 3: The motion of a microgel in an axisymmetric constriction with a tapered entrance

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Contributions: Yang Li contributed to the project by designing and carrying out all experiments, data analysis, interpretation and article writing. Prof. Ramachandran developed the theory to predict the translocation behavior of microgel. Prof. Kumacheva and Prof. Ramachandran contributed in experimental design, data analysis, and article writing.

Chapter 4: Universal behavior of hydrogels confined to narrow capillaries

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Chapter 5: A microfluidic model for in vitro studies of thromboembolism and thrombolysis

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Contributions: Yang Li contributed to the project by designing and carrying out experiments including fabrication of microfluidic device, stiffness test of microgel, occlusive and relaxation behavior of microgel, liquid flow through confined microgel, and fibrinolysis of microgel, as well as data analysis, interpretation, and article writing. Dr. Li contributed in preparation, structure and stiffness measurement of fibrin microgels, and article writing. Ms. Prince contributed in test of occlusive behavior and permeability of fibrin microgels. Prof. Weitz contributed protein molecules for fibrin gel formation and fibrinolysis. Prof. Rubinstein provided valuable suggestions in data interpretation of experimental data. Prof. Kumacheva and Prof. Ramachandran contributed in experimental design, data analysis, and article writing.

Chapter 6: Fabrication of in vitro microvascular system for study of pulmonary embolism

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Contributions: Yang Li contributed to the project by designing and carrying out all experiments, data analysis, interpretation and article writing. Ms. Pan contributed in characterization of MF-channels geometry and MF experiments of liquid redistribution. Dr. Li contributed in preparation of fibrin microgels. Prof. Ramachandran contributed in the establishment of the mathematical model to predict reflow process. Prof. Kumacheva and Prof. Ramachandran contributed in experimental design, data analysis, and article writing.
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Chapter 1
Literature review

1 Literature review

1.1 Thromboembolism in biological systems

1.1.1 Thrombosis and thromboembolism

Thrombosis is the leading cause of death in the western countries, with > 50% of population death associated with myocardial infarction, stroke, or pulmonary embolism[1]. Thromboembolism is derived from thrombosis and closely related pathological phenomena. Stroke, the second most common cause of death in the western world, as well as the single most common reason for permanent disability[2], leads to more than 15 million deaths each year. It is estimated due to the population aging, mortality rate from the stroke will almost double by 2020[3,4]. Nearly 90% of strokes are ischemic[5], mainly occurring when a clot blocks the blood supply to the neck or brain. Both thrombosis and embolism may cause ischemic stroke, and are referred to as cerebral thrombosis and cerebral embolism, respectively. The changes preceding an ischemic stroke include the hardening and narrowing of the arteries due to deposited fat, cholesterol and other substances, in the disease, known as atherosclerosis. Cerebral thrombosis happens when a thrombus develops in a narrowed artery. As a result, the blood flow to the brain is interrupted. Another common cause of stroke is embolism, when small pieces of clots, normally, from diseased arteries or the left heart valves, detach and lodge into arterial circulation and eventually, reach the brain. Cardioembolism contributes to almost 20% of ischemic strokes, and is triggered by the emboli from the left heart valves (associated with the acute myocardial infarction[6]).

Pulmonary embolism (PE) occurs when a pulmonary blood vessel is blocked by an embolus from a deep vein thrombosis in the leg. Although this life threatening pathology results in almost 60,000 deaths per year in the US alone (one of the most common causes of death in hospitals)[7], it is a difficult disease to diagnose, since the manifestations range from undetectable (e.g., shortness of breath) to fatal (e.g., shock) symptoms[8,9]. Massive, or high-risk PE causes significant damage to both the lung and the heart, such as pulmonary hypotension, myocardial infarction, cardiogenic shock and even sudden death, when heart stops pumping against the elevated resistance due to the occluded clot.
A thrombus, or a blood clot, consists of a network of fibrin fibers, and red and white cells and platelets embedded in the network. The concentration of fibrin present in normal blood is relatively low (< 10 mg/L)[10]; it is produced during blood coagulation whereby fibrinogen, the precursor to fibrin, is proteolyzed by the thrombin and eventually form an insoluble meshwork[11]. A thrombus is a healthy response to vascular injury leading to homeostasis, preventing blood loss from a damaged blood vessel. However, it can be harmful when a blood coagulation occurs in a healthy blood vessel, in the phenomenon referred to as thrombosis. Depending on the relative size of the thrombus with respect to the diameter of the vessel, a pathological of thrombus can either partially, or completely block the blood vessel, resulting in reduced or restricted supply of blood, oxygen and nutrition to downstream tissue.

The formation of thrombi is initiated by the vessel wall injury, when circulating platelets are recruited to the site of injury and activated by the exposed collagen and tissue factor from the endothelium of the vessel. Under normal conditions, both collagen and tissue factor are embedded in the endothelial matrix of the blood vessels. Upon vascular injury, exposed tissue factor mediates the binding of platelets to the exposed collagen via a specific receptor in the platelet membrane, and thus activates the platelets. The activated platelets induce aggregation of more platelets, and trigger the formation of thrombin. Thrombin not only further stimulates activation of platelets, but also converts fibrinogen to fibrin, which stabilizes the clot with the formation of fibrin meshwork[12]. A growing thrombus may break into pieces due to the shear forces imposed by the flow of blood or extrinsic intervention. Broken thrombi can affect a part of the body distant from the obstructed vessel. The lodging of a piece of the thrombus (embolus) is called thromboembolism[11] (see Fig. 1.1A).

### 1.1.2 Structure and properties of thrombi

#### 1.1.2.1 Dimensions and composition of a thrombus

The size of a thrombus (or embolus) strongly depends on the location of its forming or blocking. For example, internal carotid artery clots (the maximum width of 5 mm) were found to be larger than middle cerebral artery (MCA) clots (the maximum width of 3 mm)[13]. For even narrower branches of MCA, the majority (72%) of emboli are below 1 mm in diameter[14].
The morphology and composition of a thrombus (embolus) depends on its source, since the local microenvironment (e.g. the dynamics of flow, ionic conditions, the strength of the medium) is spatially dependent. Venous thrombi, formed by the activation of the coagulation cascade within a vein, contain a lot of erythrocytes (red blood cells) and are often referred to as “red thrombi”. Arterial thrombi, on the other hand, form owing to atherosclerotic lesions and are rich in aggregated platelets, displaying the appearance of “white thrombi”[15]. Due to the varying proportion of fibrin and red blood cells within “red thrombi” and “white thrombi”, their response to thrombolysis is different in embolic strokes[16,17]. More details in thrombolysis will be discussed in the Section 1.1.3 “Treatment of thromboembolism”.

Thrombus formation is not only a spatial but also a temporal event, and thus the thrombus composition depends on interval of time after the initiation of clotting. Ischemic time strongly affects thrombus composition during an acute coronary occlusion: fibrin content increases in a stepwise manner with time, varying from ~ 48% (< 3 h) to ~ 67% (> 6 h), whereas platelet content drops from ~ 25% (< 3 h) to ~ 9% (> 6 h)[18]. A strong effect of ischemic time on thrombus composition reveals the dynamic nature of thrombosis. “Fresh” thrombi consist of higher density of platelets in a loosely packed state. The platelets are not sufficiently activated and stabilized by fibrin[19]. With the “aging” of the thrombi, fibrin fibers keep forming with time and the platelet fraction decreases[20,21]. The premature coronary thrombi, which may form in patients who have just suffered a myocardial infarction are 50% stiffer than controls[22], due to the higher fraction of platelets in thrombi.

Despite the influence of thrombosis location and time on the composition of a thrombus, fibrin remains the major component of a blood clot in vivo. Traditionally, “white thrombi” generated in arterial blood vessels contain mainly platelet aggregates that are held together by fibrin, while “red thrombi” contain mainly red cells and more fibrin, with a lower number of platelets. Recently, it has been found that for patients suffering Acute Myocardial Infarction[18] or MCA occlusion[13], mechanically retrieved clots composed of ~ 56 or 61% fibrin, respectively. Other components of thrombi include platelets, red blood cells, white blood cells and cholesterol crystals. Notably, the fibrin content in thrombi increases with ischemic time. Fibrin also plays a crucial role in thrombi structure and mechanical properties, which determines a balance between coagulation and thrombolysis. Therefore, it is vitally important to study the mechanical properties of fibrin and the structural origins of these properties.
Figure 1.1 Origin of thrombosis, thromboembolism and properties of fibrin clot in response to strain. (A) Schematic of an embolic stroke caused by the blockage of middle cerebral artery (MCA) by an embolus originating from a thrombus in internal carotid artery (ICA). (B) Transmission electron microscopic image of negatively contrasted fibrin fibers with branching points. The band pattern with a constant periodicity of 22.5 nm is characteristic of fibrin. Scale bar = 0.2 μm. Copyright 2004 Elsevier. (C) Scanning electron microscopic image of fibers in a fibrin gel. Almost all the fibers are in a stretched state, while exhibiting twisted structure. Scale bar = 1 μm. Copyright 2004 Elsevier. (D) Schematics of fibrin polymerization. The knobs exposed in the central domain of fibrinogen with the aid of thrombin interact with holes which are always exposed at the ends of fibrinogen molecules, leading to the formation of fibrin protofibril in a half-staggered structure with a periodicity of 22.5 nm (half-length of a fibrinogen). Copyright 2005 Elsevier. (E) Strain-hardening effect of an elongated fibrin clot. A dramatically increased slope (i.e., Young’s modulus) was shown after strain is above 1.2, which is unlike collagen clot without protein unfolding mechanism shown in dashed line. The force-extension curved
(black line) can be fitted with a constitutive model by incorporating protein unfolding mechanism. Copyright 2009 American Association for the Advancement of Science. (F) The relative clot volume reduces with elongation strain (black dots), in comparison with the behavior of an incompressible material (dashed black line), the red line is from the same model applied to fit the force-extension curve. Copyright 2009 American Association for the Advancement of Science. (G) Understanding of the fibrin clot mechanics with the observed and proposed mechanism of structure changes of fibrin upon strain, ranging from centimeter to nanometer scale. Copyright 2009 American Association for the Advancement of Science. (H) Normalized FTIR spectra of unperturbed plasma clot (black line) and the same clot after 2X (green solid line), 3X (red solid line), and 4X (blue solid line) uniaxial elongation. Corresponding difference spectra (1-0, 2-0, 3-0) from the subtraction of the initial spectrum are presented as the dashed lines of respective colors. (Inset) The absorbance intensity ratio at 1622/1651 cm\(^{-1}\) as a function of strain. Copyright 2012 Biophysical Society.

1.1.2.2 Fibrinogen and its polymerization

As described in the previous section, thrombosis generally occurs in two stages, namely primary platelet activation and aggregation, and subsequent formation of fibrin strands. The precursor of fibrin is fibrinogen, a large glycoprotein with a molecular weight of 340 kDa\(^{[23]}\). Fibrinogen was one of the first biological macromolecules characterized by electron microscopy\(^{[24]}\). Fibrinogen is a water-soluble protein, 45 nm in length, with a globular central domain connecting two nodular regions at each end with \(\alpha\)-helical coiled-coils\(^{[25]}\). During clotting, thrombin, a serine protease, enzymatically cleaves the central domain (containing A and B fibrinopeptides) and liberates two small peptides, converting fibrinogen to fibrin monomer (see Fig. 1.1D). Fibrin monomers polymerize into half-staggered oligomers, due to the specific interaction between exposed “knobs” binding sites in the central domain and the “holes” that are always exposed at the ends of the protein molecule\(^{[26]}\). In the initial stage of polymerization, a two-stranded protofibril is formed with a periodicity of 22.5 nm\(^{[27]}\). The dimer structure grows linearly until a sufficient length of 600 - 800 nm is reached, followed by the lateral aggregation of protofibrils to form thicker fibers\(^{[28]}\).

Owing to the specific intermolecular interaction of lateral aggregation, the periodicity (22.5 nm) along the longitudinal direction of the protofibril is maintained, resulting in the distinct band pattern observed by electron microscopy for fibrin fibers\(^{[27]}\) (Fig. 1.1B). In the negatively contrasted specimens, areas with a higher protein density excluded stain and were bright, while regions with a lower protein density appeared darker. A constant repeating unit of 22.5 nm, or about half of the fibrin molecular length was preserved throughout the fibers between the bright and dark areas. During the lateral aggregation, protofibrils twisted around each other\(^{[29]}\), and experience stretching. Thus, exterior protofibrils were under tension, and the growth of fiber stopped when the binding energy was insufficient for stretching an additional protofibril\(^{[30]}\).
The tension experienced by exterior protofibrils agreed with experimental finding: the fibers making up the clot were almost straight (see Fig. 1.1C)[27].

Another competing and co-existing mechanism of fiber growth is branching, leading to a three-dimensional (3D) network of fibrin gel. The process of branching is critically important for fibrin function in vivo, since it results in space-filling meshwork to stabilize platelets, red bloods, or white blood cells within the fibrin gel. Electron microscopy revealed that nearly all of the branch points contained three fibril segments[27], in accordance with theoretical expectations that a trifunctional junction is the dominant branch point type[31,32]. Lateral aggregation and branching counteract each other. In other words, if lateral aggregation is favored, clots consist of thicker fibers with a lower branching density, while thinner fibers have more branching points[27,31]. The integrity of fibrin 3D network is further enhanced by covalently cross-linking it with factor XIIIa, activated by thrombin, throughout and even after the fibrin polymerization. Cross-linking occurs both within and between protofibrils, contributing significantly to the mechanical properties and fibrinolytic resistance of fibrin polymers.

1.1.2.3 Mechanical properties of fibrin

The mechanical properties of thrombi have been a subject of intensive research. A variety of types of clots formed from whole plasma, platelet-rich and erythrocytes-rich plasma have been studied. Since large-scale purification of fibrinogen was achieved more than 60 years ago, great effort has been applied to elucidate the mechanism of fibrinogen polymerization and understand its structure, as well as the mechanical properties of fibrin originating from its structure. The structural biomechanics of fibrin has gained great attention since the pioneering research in this area conducted by John Ferry[33,34]. The importance of studies of mechanical properties of fibrin stems from the following three factors: (i) fibrin mechanics determines the ability of a clot in hemostasis and thrombolysis, e.g., whether a thrombus would block or embolize a blood vessel, or then break apart; (ii) fibrin glues with controllable mechanical properties are applied in surgery and tissue engineering to stop bleeding and wound healing; (iii) cellular functions including angiogenesis and stem cell differentiation depend on fibrin mechanics[35], thus the stiffness of the fibrin-based substrate can be used to tune the phenotype and behavior of cells.
Fibrin is a viscoelastic polymer: it possesses both elastic and viscous properties. The elasticity and viscosity (or inelasticity) is reflected by its reversible and irreversible deformation, respectively. Taken together, the viscoelastic properties of fibrin determine the response of clots towards blood flow (e.g., complete or incomplete structural recovery after the shear deformation due to the blood flow) and complex geometry of the microvascular network in vivo (e.g., embolization or obstruction). Furthermore, the viscoelasticity of fibrin also determines the response of clots to therapeutic treatment, such as administration of fibrinolytic agents.

The viscoelasticity of fibrin is commonly characterized by shear rheometry, where controlled shear stress is exerted on a fibrin clot in a torsional manner, and the resulting strain is recorded. The stress-strain relationship is generated to determine elasticity (the shear storage modulus G’), and viscosity (the shear loss modulus G’’). The terms “storage” and “loss” represent saved and lost energy during clot deformation, respectively. In a tensile rheometer, the elasticity can be measured by recording the strain of the fibrin clot due to the applied stretching stress. The plotted stress-strain curve is then used to determine the elastic modulus of the fibrin clot from the slope curve in the initial linear elastic region. In both types of rheological measurements, a higher value in modulus refers to a larger contribution of respective modulus component in the viscoelastic properties of a fibrin clot.

When the extent of deformation of a fibrin clot is small and the strain frequency is low, Hooke’s law applies to the stress-strain curve, indicating non-existence of inelastic component in clot deformation. At higher rates of deformation, however, the viscous component becomes apparent and grows rapidly with rates. In general, the elastic component is an order of magnitude larger than the inelastic one. The reported elastic moduli of fibrin clots vary in the range from 0.1 to 1,500 Pa, depending on the clot structure. One remarkable feature of fibrin compared to other biomaterials is that, the storage modulus G’ of a fine clot with a dense fibrin network is almost independent of frequency over almost 8 decades. Such constant stiffness over time suggests self-healing properties of fibrin, which most likely, are related to reversible knob-hole interaction across the fibrin, against the traditional perception that the fibrin monomer interaction within a protofibril is irreversible. The invariable stiffness may preserve the integrity of blood clots under various rates of applied stress in vivo due to pulsatile flow of blood and varying pathological conditions, further emphasizing the physiological importance of fibrin viscoelasticity.
When the strain is above 1.2, during tensile rheometry, a linear stress-strain relationship of can no longer be maintained and the slope of the curve, i.e., the elastic modulus of the fibrin clot, exhibits one order of magnitude increase in comparison with its original value (Fig. 1.1E)\(^{[41,42]}\). The non-linearity in stress-strain curve is called strain hardening or stiffening. It was also observed for fibrin gels under shear deformation\(^{[35]}\).

In addition to significantly increased stiffness, ~2.7-fold of length extension relative to its rest length was also found before material failure during tensile stretching\(^{[42]}\), and the value is comparable to single-fiber extensibility of fibrin\(^{[43]}\). Moreover, a significant volume reduction of fibrin clot was observed upon stretching, with less than 10% of its rest volume has preserved for strain of 1.5, inducing an ~10-fold fibrinogen concentration within the clot (Fig. 1.1F)\(^{[42]}\). The sudden increase in stiffness is uncommon and may be physiologically important, since it allows fibrin clots to be compliant at small strain and protect clot integrity under higher strain.

In addition to shear and tension, strain-hardening effect was also observed in compressive experiments on fibrin gels. A more complex tri-phasic stress-strain response was found in recent studies\(^{[44,45]}\). When a fibrin clot was exposed to increasing compressive strain, gradual softening was followed by dramatic stiffening of the gel. The initial softening correlated with fiber buckling and bending, whereas strain hardening was associated with an increase in fibrin network density and crisscrossing of fibrin fibers.

Despite experimental evidence over decades\(^{[41]}\), theoretical explanations on the origin of fibrin elasticity, especially, strain-hardening effect, have only recently been developed and described on multiple length scales\(^{[42]}\). In this theory, in addition to the behavior of fibrin in the scale of entire clot (10\(^{-2}\) m), as described earlier, structural changes underlying fibrin elasticity were categorized into three groups, namely, for the network, for individual fiber, and on the molecular level.

At a network length scale (10\(^{-5}\) m), unperturbed fibrin gels contain randomly orientated fibers with a relatively larger space between them, as shown in the scanning electron microscopy (SEM) image (Fig. 1.1G). Upon tensile strain, however, the fibrin structure become ordered, with fibers aligning along the stretching direction. The re-orientation of fibrin network was also observed for fibrin gels under compressive strain\(^{[44]}\), where fibers rearranged and exhibited crisscrossing.

At the fiber level (10\(^{-6}\) m), upon gel stretching fibers become thinner, are spaced closer, and even bundled together according to SEM images\(^{[42,46]}\). The bundling and thinning of fibers contribute to the shrinkage of
the entire fibrin clot. In response to compressive or shear strain, unlike in tensile stretching, individual fibers start to bend and buckle in the direction of deformation\cite{44,47}, incurring gradual decrease in stiffness as described earlier. Upon stress for a time period of 1 h order or less, fibrin clots made from thicker fibers exhibit more irreversible deformation than those made of thinner fibers\cite{39,40}. The creep behavior is associated with the slippage of protofibrils past each other within a fiber\cite{27}. Thicker fibers are composed of a higher number of protofibrils, thus stronger lateral aggregation between protofibrils. Once the aggregation is disrupted and slippage occurs upon strain, it is more difficult for fibrin molecules in thicker fibers to establish a new interaction across the protofibril. Therefore, more irreversible deformation leads to fluid-like mechanics in fibrin gels with thicker fibers.

At a molecular level (in the nanometer scale), gel extensibility, volume reduction and strain-stiffening are attributed to the unfolding of fibrin molecules, namely, a change from $\alpha$-helix to $\beta$-sheet structure during fibrin stretching and subsequent molecular unfolding (Fig. 1.1G). Due to uniaxial stretching of the fibrin clot, hydrophobic side chains within proteins become exposed to water and begin to interact with each other, leading to water expulsion, as well as the strain-hardening\cite{42}. The $\alpha$-$\beta$ conversion was first observed by low-resolution wide-angle X-ray scattering (WAXS) in 1943\cite{48}. Recent experimental evidence was obtained by stain polarimetry\cite{49}, where unperturbed fibrin gel was stained with Congo red and placed under polarized microscope. Upon 3-fold stretching, the color change of fibrin under strain revealed the secondary structure change, since the colored congophilic material is associated with stacks of $\beta$-sheets\cite{49}. More recently, $\alpha$-$\beta$ conversion was proved, with higher specificity, during extension and compression of fibrin clot, characterized by Fourier Transform Infrared Spectroscopy\cite{50}. As shown in the dashed lines of the difference spectra (spectrum of fibrin clot under certain strain subtracted from the initial spectrum at zero strain) in Fig 1.1H, intensity at 1622 cm$^{-1}$ is assigned to $\beta$-sheet structure, and intensity at 1651 cm$^{-1}$ to $\alpha$-helix structure. With increasing strain, a higher ratio between intensities of 1622/1651 cm$^{-1}$ was observed, indicating the secondary structure transition. Moreover, partial recovery of the protein unfolding for fibrin clots, where the ratio of intensities 1622/1651 cm$^{-1}$ partly decreased after the stress was released. To explain the reversibility, $\alpha$-$\beta$ conversion was considered as a biphasic process with elastic and inelastic components. The elasticity was associated with the force-induced switch from $\alpha$-helix to $\beta$-sheet, whereas the inelastic component was related to the irreversible intermolecular aggregation of $\beta$-sheets. Such mechanism agreed
with the experimental findings that the degree of reversibility correlated directly with strain, namely, a larger elongation incurred more irreversible deformation of fibrin clots\(^{[50]}\).

As discussed above, the remarkable mechanical properties of fibrin gel originate from its multiscale hierarchical structure, which can be tuned by manipulating the concentrations of fibrinogen, thrombin, calcium ion, or Factor XIIIa during clot formation. Due to the complexity of the polymerization process, the effect of a particular component on fibrin architecture cannot be clearly resolved without considering the contribution of other factors\(^{[31]}\).

### 1.1.3 Treatment of thromboembolism

Thrombosis is the leading cause of death in the western countries, which results in > 50% of population death associated with myocardial infarction, stroke, or pulmonary embolism\(^{[1]}\). Therapeutic methods to treat thromboembolism have been extensively studied and developed, and they can be mainly classified into three types, namely, reperfusion with intravenous (IV) tissue plasminogen activator (t-PA), endovascular therapy (e.g., intra-arterial thrombectomy and stent-retriever technology), and the combined therapy using two methods above. The first type of therapy involves the fibrinolytic digestion of thrombus or embolus, whereas the second type of therapy relies on the mechanical manipulation to remove clot in a faster but invasive method. The three methods have all been clinically adopted on both arterial and venous thromboembolisms. The following subsections describe their applications in two fatal diseases, i.e., acute ischemic stroke and pulmonary embolism.

#### 1.1.3.1 Intravenous (IV) fibrinolysis

Currently, the use of IV recombinant tissue-type plasminogen activator (alteplase; rt-PA) is the only proven reperfusion therapy for acute ischemic strokes, and its clinical efficacy is strongly dependent on the period between the onset of stroke and the administration of rt-PA. The treatment has to be initiated within a relatively short time window (< 4.5 h), in order to improve the clinical outcome\(^{[51,52]}\), although rt-PA may be still beneficial when the administration is within 12 h after the onset of the symptoms\(^{[53]}\). Due to its
fibrin-specific behavior, rt-PA is efficient in dissolving clots, however, it also increases the risk of reocclusion (occurrence in 5 - 15% recanalized arteries)[54,55], most likely because thrombin is formed from activated circulating prothrombin during fibrinolysis. Thrombin will catalyze the transformation of fibrinogen into fibrin[54]. As a result, non-fibrin-specific antithrombotic agents (heparins) are usually administered concomitantly with fibrin-selective agents[56].

Significantly improved clinical benefit of rt-PA has been confirmed by several meta-analyses based on pooled data of thrombolytic treatment[57–59], in which a notable increase in patient survival and independence in daily activities resulted from thrombolytic therapy. In one of the studies, pooling data from an aggregate of 2775 patients surveyed in six large randomized trials in multiple research centers[59], the odds of a favorable, 3-month outcome after rt-PA administration compared with controls was found to continuously decrease from ~ 3.5 times to ~ 1.0 time with the period of time between rt-PA administration and stroke onset being from 60 to 360 min, suggesting that the time after stroke onset is crucial to IV treatment efficacy. In patients with massive pulmonary embolism, in which a hemodynamic instability (sustained low blood pressure, low heart rate with signs of shock) was detected[60], thrombolytic therapy also resulted in profound reduction in recurrent pulmonary embolism or death[61].

The proposed scheme of fibrinolysis by t-PA is shown in Fig. 1.2A. The key step of t-PA regulation in vivo is its co-localization with circulating plasminogen on a fibrin surface, whereby a ternary complex, fibrin-plasmin(ogen)-t-PA is formed to stimulate the generation of plasmin (Fig. 1.2A)[62]. The ternary complex leads to an increase of t-PA activity of $10^2 – 10^3$ fold[63]. The activation of plasminogen to plasmin is further enhanced by the initial cleavage of fibrin, through which kringle domains containing lysine binding sites in both plasminogen and t-PA bond with C-terminal lysine residues exposed from cleaved fibrin[64–66]. This positive feedback mechanism promotes the lysis of fibrin with enhanced binding of plasminogen and t-PA, and eventually, the generated plasmin digests the whole blood clot through a series of biodegradable products (Fig. 1.2B). In a typical fibrinolysis process with the addition of t-PA, a lysis front can be observed as a narrow zone of concentrated plasminogen (indicating aggregation and activation of plasminogen) moving inwards the clot as characterized by fluorescence microscopy[67–69].
Figure 1.2 Mechanism of fibrinolysis and the dependence of fibrinolytic resistance on the elongation strain. Proposed mechanism for the initiation (A) and propagation (B) of fibrinolysis. A ternary complex of fibrin-plasmin(ogen)-t-PA forms to stimulate the generation of plasmin, which results in the digestion of fibrin with the subsequent series of fibrin degradation. Copyright 2015 International Society on Thrombosis and Haemostasis. (C) Lysis of non-stretched (first row) and 2X stretched (second row) of fibrin clot with confocal laser microscopy. Green-fluorescent-protein-labeled t-PA was added to red-fluorescent-protein-labeled fibrin, and individual color channel was applied to image respective material, with the third panel being the overlay of green and red channels. Time was counted from the addition of t-PA. Scale bar = 50 μm. Copyright 2011 International Society on Thrombosis and Haemostasis. Fibrinolysis characterized by volume (D) and mass (E) loss with time is shown for the plasmin digestion of fibrin clots under uniaxial stretching. (D) Normalized radius (radius ratio between the rest of fibrin gel and initial fibrin gel) decreases with time due to plasmin digestion, and the rate of lysis (slope) decreases with an increase in strain. (E) Rate of mass removal versus strain, by considering the observed volume reduction and known fibrin density. Copyright 2012 American Chemical Society.

Although the binding front of fibrinolytic agent localizes in a narrow area on the surface of a fibrin clot, the resistance of fibrin to lysis strongly depends on its structure\textsuperscript{[62,67,70]}. Two seemingly contradictory phenomena are that fibrin clots made of thin fibers are dissolved at a slower rate compared to the clots made of thick fibers although individual thin fibers are easier to cleave than thick ones\textsuperscript{[67,71--73]}. This discrepancy
can be explained by the compactness-induced differences in t-PA binding\textsuperscript{74} and permeation\textsuperscript{75}, as well as plasmin diffusion\textsuperscript{62} within the 3D network of the fibrin clot. During the formation of a fibrin gel, thin fibers always form dense branching points and thus have a smaller pore size. These features significantly limit the movement of t-PA and plasmin molecules within the meshwork, rendering lower lysis rate of the clot made of thinner fibers. Such mechanism agrees with clinical findings, in which reduced clot permeability and higher fibrinolytic resistance is associated with increased fiber density\textsuperscript{22,76}. Extensive research on other thrombotic disorders, including venous thromboembolism\textsuperscript{77}, stroke\textsuperscript{78}, or peripheral arterial disease\textsuperscript{79} has also proved the relationship between fiber density and lysis rate of the blood clots.

The mechanism above can be extended to explain, to some extent, recent observations of a stronger resistance of a stretched fibrin gel to lysis\textsuperscript{80–82}. Due to the varying magnitude of shear stress imposed by blood flow on a thrombus in vivo, mechanical forces vary dramatically at different positions of an individual stenosis\textsuperscript{83}, and thus the architecture of the fibrin network varies from clot to clot, and even from the interior to the exterior of a single clot\textsuperscript{80}. 40\% of the examined thrombi exhibited orientated exterior fibrin fibers with the interior fibers being randomly orientated, and the other 60\% thrombi did not show any alignment of fibers throughout the whole clot\textsuperscript{80}. The orientation of fibers can be attributed to the shear flow along the exterior surface of the thrombus\textsuperscript{84,85}. Therefore, the heterogeneous fibrillar architecture of fibrin clots motivated studies of the impact of strain on the fibrinolytic resistance of fibrin gels. In the first study of dissolution of fibrin under stretching, the initial stage of fibrinolysis (plasminogen activation) was greatly hampered, as reflected by the observation that the t-PA lysis front was completely blocked within an hour on the two-fold stretched fibrin gel (a less sharp and thinner interfacial layer of FITC labeled t-PA), compared to unperturbed gel (see Fig. 1.2C). In addition to the proposed mechanism above that t-PA\textsuperscript{74} and plasminogen\textsuperscript{75} binding would be restricted due to higher packing density of fibrin fibers after elongation\textsuperscript{42}, reduced binding sites for these two molecules upon protein unfolding may also account for the deficiency of t-PA activation\textsuperscript{80,81}. Efficiency of the secondary stage of fibrinolysis, namely, plasmin digestion, also greatly reduced with the applied elongation strain\textsuperscript{82}. The rate of fibrinolysis, characterized by the reduction of normalized radius of fibrin continuously decreased with strain (Fig. 1.2D), with up to \sim 10-fold drop in mass removal rate with the strain of 1.8 (Fig. 1.2E). Furthermore, the diffusivity perpendicular to the elongation decreased with increasing strain, which agreed with the proposed correlation between enhanced fibrinolytic resistance and impeded plasmin permeation. To note, such studies have been limited to the
uniaxial elongation of a fibrin clot, and therefore, additional research is needed to understand 3D deformation of clots *in vivo*.

### 1.1.3.2 Endovascular therapy

Despite the efficacy of thrombolytic treatment, rt-PA may cause side effects such as bleeding\[^{86,87}\]. Such limitation renders up to 60% of patients with massive pulmonary embolism not receiving systemic thrombolysis\[^{88}\]. Therefore, for such patients or for patients with poor response to rt-PA, endovascular therapy (EVT) is a valuable alternative. Additional advantages of endovascular therapy (or catheter-based surgery) over thrombolysis reside in less restrictive time window (up to 8 h)\[^{89}\] and its efficiency in removal of mature thrombi with high resistance to rt-PA\[^{2,90}\].

Currently, two generations of mechanical thrombectomy devices have been adopted for endovascular therapy (EVT) in acute ischemia stroke. The first-generation of Merci retriever was approved in 2004 by the United States Food and Drug Administration. This device consists of a long thin wire with a helical coil at its end, and is usually combined with a silicone balloon guide catheter during application. The balloon is inflated with air to temporarily arrest the blood flow upstream of the clot. Following its straightening configuration for fitting in the catheter, the helical coil then becomes coiled, again, in order to trap and aspirate the clot under negative pressure\[^{90,91}\]. A device of a different type is used for clot fragmentation along with aspiration\[^{92}\]. Catheter-based techniques have been used for the treatment of pulmonary embolism in the forms of thrombus fragmentation\[^{93}\], rotational thrombectomy\[^{94}\], and rheolytic thrombectomy\[^{95}\].

Optimized, 2nd-generation devices, known as stent retrievers, are preferred to balloon-based stents due to the decreased risk of vessel dissection and rupture\[^{96}\]. The stent self-expands within a blood vessel to push the clot against the vessel wall and recanalize the flow instantaneously, followed by the retraction of the folded stent with the clot which has already been trapped within the stent\[^{97}\]. Stent retrievers are considered as a major advance in EVT, reflected by a higher successful rate of recanalization with lower possibility of hemorrhage\[^{98}\].
1.1.3.3 Combined therapy

The selection of a particular method of treating thromboembolism (IV fibrinolysis vs. endovascular therapy) is not an easy decision. Three recent randomized trials of superiority of EVT vs. IV fibrinolysis published showed no significant benefit of using EVT compared to systematic fibrinolysis\[99-101\]. More recent evidence from randomized controlled trials, however, revealed that EVT is more beneficial over rt-PA administration alone, in terms of improved reperfusion, early neurologic recovery, and favorable function independence\[102,103\]. The application of stent-retriever device largely contributed to the increased benefit of using EVT over both IV fibrinolysis and previous thrombectomy devices\[103\].

Although further large randomized controlled trials are needed to conclude the superiority of one method over the other, the best medical therapy to thromboembolism remains the combination of these two methods\[97\]. When applying stent-assisted IV fibrinolysis in acute ischemic stroke, 100% recanalization rate was achieved in 20 patients, with only 1 (5%) symptomatic hemorrhage in treated patients\[98\]. A combination of catheter-directed thrombolysis with traditional mechanical embolectomy also increased the overall clinical success rate from 81% to 95% in the treatment of pulmonary embolism\[104\].

1.2 Studies of thromboembolism in vitro

In spite of the intuitive cognition that the relationship between the size of blood vessel and thrombi (emboli) could be an important indicator for the risk and the location of thromboembolism, the data confirming this hypothesis is scarce\[105\]. In this section, experimental and theoretical studies on their mechanistic effect on the distribution of thromboemboli within microvascular network (mainly, bifurcations) are reviewed.
1.2.1 Experimental studies

Two types of emboli have been used to model embolic stroke, namely, autologous emboli and surrogate emboli. In the former approach, spontaneously formed thrombi or thrombin-induced thrombi from autologous blood were used\textsuperscript{[106,107]}. Although important correlation between obstruction of blood flow, infarct volume and location, and the outcome of thrombolysis and the origin, e.g., the formation and composition of the emboli was observed in such studies\textsuperscript{[108,109]}, the advantage of this model resided in the inherent closeness between autologous thrombi and blood clots \textit{in vivo}. In terms of statistical investigation of flow trajectories of emboli within cerebral system, this type of model produces variable infarctions\textsuperscript{[110]} and embolism is unpredictable\textsuperscript{[111]}.

In terms of controlling the shape, size, and mechanical properties of the real clots, surrogate emboli from a variety of sources have been adopted to investigate the dynamics of clot motion through an arterial bifurcation in cerebral microvascular system with the aim to achieve better perception of emboli distribution in the brain. In one of earlier studies\textsuperscript{[112]}, glass microspheres with a size range of 90 - 210 $\mu$m were perfused into brains of cadavers. Bigger 150 - 210 $\mu$m-size particles were preferentially localized in the most distal extensions of the main arterial trunks. Particles less than 150 $\mu$m in diameter were randomly distributed in all vascular regions. The explanation of such size-dependent trajectory, is provided by the Fahraeus-Lindqvist effect\textsuperscript{[113]}. Particles prefer to stay in the center streamline, resulting in few or none particles in the most peripheral streamline; and only the peripheral particles enter the branches. Moreover, the bigger the particles are, the more often they remain in the center streamline, thereby rendering their bypassing most of branches, and propagating in the watershed zone of the brain.

Polymeric microspheres with designed size ranges, were also applied in animal studies, for example, agarose spheres were injected into an internal carotid artery (ICA) of monkeys and their traffic in circumferential and penetrating arteries were measured\textsuperscript{[114]}. Small spheres (30 and 70 $\mu$m) randomly flew into both regions and could lead to produce lacunar infarction, while bigger spheres (92 $\mu$m) entered peripheral, rather than penetrating arteries. In an assumption that the size of the majority of emboli \textit{in vivo} is larger than or at least as large as the size of penetrating arteries, the observed phenomena explained that most patients with embolic source of stroke would suffer cortical, rather than lacunar infarction.
Polystyrene microspheres with dimensions from tens of micrometers to several millimeters\textsuperscript{[115-117]} have been selected to study the redistribution of emboli at the terminal bifurcation of the ICA, namely, in either narrower anterior cerebral artery (ACA) or wider middle cerebral artery (MCA). It was thought that the relative flow rates in these two paths would determine the microsphere redistribution, however, clinical evidence suggested that emboli from ICA are >20 times preferred resting in MCA to ACA\textsuperscript{[118]}, that is significantly higher than the ratio of flow rate determined by the two branches. An \textit{in vitro} study based on a Y-shaped bifurcation model demonstrated the distribution of polymeric emboli was beyond its flow ratios between ACA and MCA, with a tendency that larger emboli prefer entering a wider bifurcating branch. Such preference was enhanced with the application of pulsatile flow\textsuperscript{[115]}. Rather than using a Y-shaped junction, a patient-specific, transparent 3D replica made from silicone has been recently used\textsuperscript{[116,117]}. The observed distribution of polystyrene spheres was consistent with that obtained for MCA/ACA infarcts, that is, ~95% of the spheres resided in MCA. The distribution began to correlate with the flow ratio between two branches when the sphere size was below 200 $\mu$m\textsuperscript{[116]}.

Natural clots with more relevance to real thrombi, but, with less control in size and shape, have been also been utilized\textsuperscript{[119,120]}. Fragmented plaques, after filtering with sieves of 60, 100, and 200 $\mu$m, were injected into rats. Small emboli (60 and 100 $\mu$m) with irregular shapes blocked arteries and resulted in lacunar infarction. In addition, calcified emboli led to infarction with a significantly larger probability compared to control\textsuperscript{[119]}, thus suggesting the influence of clot rigidity on infarction. In a more recent study, biomimicry clots from the hemolymph of a crustacean species, which is analogous to the composition to mammalian blood, were perfused into a 3D patient-specific cerebral model\textsuperscript{[120]}. The distribution of the clots with a cylindrical shape (3.2 mm by 20 mm) was investigated, along with the deformation of clots. The elongation ratio was relatively weak (0.84 $\pm$ 0.53) when the radial compression was between 1.1 and 4, indicating compression, instead of elongation, dominates the deformation of emboli in a patient-specific physical model.

1.2.2 Theoretical studies

In addition to the animal studies and \textit{in vitro} studies described above, several computational studies of embolus trajectory within microvascular system have been conducted\textsuperscript{[116]}. Due to the complexity of human
cerebral arteries, a minimal model simulating emboli transit through a single portion of the human cerebral vasculature was recognized as a realistic starting point\textsuperscript{[121]}. In an earlier attempt, a fractal network of symmetrically bifurcating vessels with decreasing diameters along with descending generation of vessels was used to study the motion of emboli via Monte Carlo simulation\textsuperscript{[121,122]}. By assuming that the movement of an embolus stops when it encounters a blood vessel with the same diameter, the origin of stroke due to embolization has been analyzed. A specific combination of emboli size and embolization rate was found to induce a fast transition from freely-perfused to completely blocked arteries. Moreover, the impact of emboli on stroke was simulated. It was found that an intact, large embolus would be much more fatal than the broken fragments from the same embolus. In another study, an asymmetric branching model with different sizes of two daughter vessels was employed\textsuperscript{[123]}. In addition to a similar finding that an increase in embolus dimensions and rate increases the possibility of arterial blockage, it predicted that a narrower daughter vessel would be occluded at a lower rate, since emboli preferentially occlude larger branching vessels.

A step advance in theoretical modeling was achieved by introducing stenosis into simulations\textsuperscript{[6,111]}, since partially occluded arteries were found to strongly impact infarction\textsuperscript{[124]}. According to the analysis of the motion and deformation of a clot within a constricted artery, the size of an embolus, rather than the stenosis severity, has greater effect on its stress level during embolism\textsuperscript{[111]}. With the same approach, by incorporating a 3D patient-specific model, the trajectory of emboli with various size and density was simulated, and a reduction of embolus flow velocity was observed with either embolus size, or density\textsuperscript{[6]}.

As described in the previous section, the preferential distribution of emboli in MCA, compared to ACA, was experimentally observed. More recent computational modelling has been applied to simulate this effects in a computerized model reconstructed from anatomical images\textsuperscript{[105,125,126]}. In one of the studies, cardiogenic emboli from the ICA source were found to have a distribution, which partially contradicted \textit{in vitro} results. The results indicated that medium size emboli (~ 1 mm) prefer to enter ACA, while only large emboli tend to enter MCA. In another study\textsuperscript{[125]}, the distribution of embolic particles with various size and density was investigated. Larger or lighter emboli tend to travel into larger branches (MCA), which agreed with experimental findings. This study again emphasized that the local branch patterns dramatically affect the distribution of emboli into MCA or ACA bifurcation. More recent research considered the effect of mechanical properties of emboli on the thromboembolism\textsuperscript{[126]}. With an increase in embolus stiffness, a
higher possibility existed to enter larger arteries (MCA). Such simulation may be applied not only to understanding of the origin of stroke, but also to clinical decisions on treating thromboembolism.

To summarize, in vitro studies of thromboembolism are restricted either by the control of properties (size, shape, and mechanical properties, etc.) or by the biological relevance of the adopted emboli. In theoretical studies, rapid development of detailed simulations of emboli and microvascular system, however, the lack of experimental data limits the range of conclusions based on the computational models. Therefore, a bridge between these two studies is required in the form of an experimental model of thromboembolism with a balance between control in dimension, microscale morphology, as well as viscoelasticity of artificial emboli and their biological relevance.

1.3 Modelling vascular systems

The replication of the well-defined three-dimensional (3D) microvascular architecture found in native tissues is of great importance in microvascular research and tissue engineering. A microfabrication technology that can provide a vascular microcirculation system including arterioles (250 \( \mu \text{m} \geq \text{diameter} \geq 8 \mu \text{m} \)), capillaries (diameter < 8 \( \mu \text{m} \)), and venules (250 \( \mu \text{m} > \text{diameter} \geq 8 \mu \text{m} \)), thus mimicking the complex architectures of in vivo blood vessel network\(^{[127]}\), would be invaluable in generating a systematic understanding of blood flow, flow-induced distribution of blood particles, cell growth and alignment, thrombosis and embolisms. To this end, many efforts have focused on the fabrication of 2D or 3D microfluidic channels to provide an artificial microvascular system. Two critical attributes of these artificial microvascular system are the cylindrical nature of constituent microchannels, and the 3D nature of the network\(^{[128]}\). In this section, we highlight recent advances in the microscale fabrication techniques which are capable of providing in vitro models from single blood vessels towards 3D microvascular network. In terms of material, we classify the modelling paradigms into silicon, polymer and hydrogel categories, and we also overview the rapidly progressing 3D printing in constructing complex biological tissues.
1.3.1 Silicon-based vasculature

Silicon- and glass-based structures have been applied to fabricate microfluidic devices over 20 years due to their adoption within microelectromechanical systems (MEMS) and micromachining. Wet or dry etching method followed by the predefined patterning on top render a controllable geometry profile into the bulk silicone/glass substrate. In wet chemical etching, usually implemented with hydrofluoric acid, the carved profile depends on the crystal orientation of silicon/glass, but is usually isotropic in nature. Dry plasma/gas silicon etching provides flexibility in choosing the etching direction, and allows higher etch rate and selectivity between mask and substrate. Inductively coupled plasma (ICP) is the most efficient plasma source for dry etching, and is capable of deep anisotropic silicon etching with high etch rate; however, the method is more expensive than wet chemical etching.

Owing to the innate non-transparency of silicon, the deficiency of visual characterization largely limits its application in mimicry of microvasculature. Instead of inner structure throughout the silicon substrate, interfacial pattern of the microfluidic channels along the surface of the substrate was achieved by xenon difluoride etching of silicon wafers under masks\textsuperscript{[129]}. The isotropic etching led to channels with semi-circular cross-sections on the silicon wafer, and a four-generation silicon structure with varying height in each generation was obtained with a relatively complex procedure involving six masks and six independent etching processes. Moreover, the opaqueness of silicon necessitates the sealing of the channels by a transparent glass slide, resulting in a semi-circular cross-section for the channel, and not the desired circular one.

In comparison with silicon, the transparency of glass guarantees a feasible real-time inspection of the blood flow within a glass capillary with well controlled dimensions. Flow behavior of either whole blood\textsuperscript{[130]} or individual blood cells was investigated within the cylindrical glass capillary with varying dimensions. For example, the velocity field for the flow of a suspension of red blood cells (RBCs) inside a glass capillary was obtained by tracking the motion of RBCs with confocal micro-Particle Image Velocimetry (PIV) method\textsuperscript{[130]}. The effect of shear stress on the individual cell attachment to the inner surface of the capillary was examined in protein-coated glass capillaries\textsuperscript{[131]}. Three-dimensional hollow MF structures inside glass substrate were achieved by nanosecond\textsuperscript{[132]} or femtosecond laser\textsuperscript{[133]} writing with subsequent selective chemical etching, and has been implemented in detection and manipulation of single cells\textsuperscript{[134]}. 
Although cost-efficient, glass capillaries have rigid walls and difficulty to incorporate complex geometries. These limitations have been overcome by novel, low cost alternatives based on polymers over the past 15 years.

1.3.2 Polymer-based vasculature

Polymers show several major advantages over silicon or glass, including a wide range of material characteristics, low cost of manufacture, biochemical compatibility, and resistance to chemical corrosion\[135,136\]. Due to the extensive application of poly(dimethyl-siloxane) (PDMS) in this area of study, we have split the discussion in this section into PDMS and non-PDMS based artificial microvasculature.

1.3.2.1 PDMS

PDMS is a common structural material with optical transparency, non-toxicity, chemical stability, and biocompatibility. In addition, its elasticity (360 - 870 kPa) is within the range of elasticity of blood vessels\[137\] and also controllable, to some extent, by tuning the mixing ratio between Dow Sylgard 184 polymer and curing agent prior to polymerization. Compared with most other polymers, PDMS is both oxygen and water permeable, which enhance its suitability in biological and biomedical applications. PDMS has become the predominant choice as the material for bio-MF chips since the inception of soft lithography technology\[138\]. A typical soft lithography procedure involving PDMS stamps is described as follows. A patterned structure of an epoxy-based negative photoresist (SU-8) is first fabricated on a silicon wafer in the cleanroom. SU-8 is spin-coated on a silicon wafer to the desired thickness, and exposed to UV light through a photomask with the desired microchannel pattern. The exposed SU-8 crosslinks and remains after development, while SU-8 covered by the photomask is washed away after development. The rigid SU-8 pattern is called master. PDMS is then poured over the master and subjected to curing at a predefined curing temperature. After curing, the PDMS stamp is then peeled off from the master, and is ready to assemble into a MF chip with a glass or PDMS substrate. The advantages of PDMS related soft lithography...
reside in ease of designing of complex structures, direct molding, repeatable usage of masters, excellent sealing properties, and convenient incorporation of fluidic interconnects\textsuperscript{[135]}.

PDMS MF channels have been extensively applied to investigate traffic of blood cells within the polymer capillaries, especially the transit of a blood cell through a narrow PDMS segment with diameter smaller than the diameter of the blood cell; this is a topic highly relevant to the thesis. The stiffness of diseased red blood cells is known to be much higher than healthy ones, and a narrow segment within the MF channel serves as a model of blood capillary, allowing a mechanical screening of red cells. MF channels with single constriction\textsuperscript{[139–142]} or multiple parallel constrictions\textsuperscript{[143,144]} were adopted to study the flow behavior of infected red blood cells. The dependence of transit time\textsuperscript{[139,141–143]}, transit trajectory\textsuperscript{[140]}, clogging states\textsuperscript{[144]} on the diseased stage of the red blood cells were established on such MF platforms in a relatively high throughput manner. A direct observation of white blood cell deformation, transit, and relaxation through a constriction within a PDMS channel was reported, and a sequence of constrictions with short spacing was designed without allowing the white blood cells to fully recover their original shapes. Consequently, the cells were found to cross the first constriction in about 25 secs, but in only 0.5 sec for all subsequent constrictions\textsuperscript{[145]}. By modeling the partial obstructed blood vessel with a PDMS MF channel with a narrow segment, high shear stress was induced by the stenosis, and led to the breaking up of the micro-aggregates into nano-components\textsuperscript{[146]}. The biomimetic stenosis offers a potential treatment to thrombosis or embolism, since a partially obstructed blood clot normally causes abnormally high fluid shear stress \textit{in vivo}. More complicated physiologically realistic vasculature with numerous bifurcations was also fabricated to mimic muscle\textsuperscript{[147]}, lung\textsuperscript{[148]}, and even a part of capillary system\textsuperscript{[149]}.

The numerous advantages of the PDMS-based MF channels are offset by one key drawback - the rectangular cross-section inherent from soft lithography by using SU-8 negative photoresist. The upright UV-exposure induces cross-linking of SU-8, and renders its stabilized cuboid feature as a master, as well as the subsequent cuboid feature for the PDMS stamp. The heterogeneous seeding of cells in the sharp corner regions\textsuperscript{[150]} induces significant variations in cell physiology\textsuperscript{[151,152]}. Wall shear stresses vary dramatically at these locations\textsuperscript{[153,154]}, leading to potential impacts on flow-based studies\textsuperscript{[155,156]}. Moreover, the occluded biological microparticles (blood cells, emboli, etc.) cannot fully conform to the rectangular cross-section, and the resultant leakage of liquid flow through the corners significantly compromise the capability to mimic blood vessels. This limitation has been overcome recently, and PDMS MF channels
with circular cross-section have been fabricated using mainly the following three strategies: reshaping, sacrificial template and photoresist reflow.

The mechanism of reshaping of PDMS channel with original rectangular shape into circular shape is to use the PDMS surface tension before it is cured and solidified. One of the reshaping protocols originated from our research group\cite{157}, where a solution of PDMS prepolymer and curing agent in hexane was introduced into the microchannel of a rectangular cross-section, followed by a nitrogen gas stream. The system was then heated to polymerize the PDMS and evaporate hexane. Notably, the pressure of the injected nitrogen determined the shape and size of the resultant cross-section, and a circular shape can be achieved by adjusting the parameters into appropriate values. Following this method, we have investigated the flow behavior of an individual agarose microgel through PDMS MF channel with circular cross-section\cite{158,159}, and this work will be elaborated in Chapters 3 and 4 of this thesis. In a more recent study, circular cross-sectional PDMS channel, prepared by a similar method, with a diameter of less than 10 μm served as human capillary model to study the circulation of tumor cell clusters\cite{160}. In contrast to the previous belief that tumor clusters are too large to translocate through constrictions in vivo, the micro-aggregates of tumor cells exhibited a rapid and reversible “unfolding” behavior (Fig. 1.3A and B), leading to a smooth passage of tumor cells in sequence. The phenomenon of easier passage of tumor cells shown in this work suggests that the dissemination of tumor cell clusters to downstream organs would be responsible for their metastatic potential. Another research investigated the flow-induced rupture of circulating tumor cells (CTCs) at a micropillar-based bifurcation, and metastatic CTCs were found less prone to mechanical fragmentation at the MF junction\cite{161}. This study proposed the capability of MF channels to phenotype CTCs based on their deformability.

Another reshaping method utilizes the reshaping capability of partially cured PDMS\cite{162}. The “gel-stage” PDMS possesses higher viscosity and surface tension, and will remold into semi-circular shape upon contact with a PDMS mold with rectangular shape followed by the complete curing. By using the fully cured semi-circular shaped PDMS as a mold, a second piece of partially cured PDMS substrate will be transformed into semi-circular and cured together with the first semi-circular piece, resulting in a sealed PDMS MF channel with circular cross-section. The key procedure in this work is the partial curing of PDMS oligomer solution, which strongly affects the capability and extent of reshaping. Partial curing also allows the preparation of 3D crossover network of PDMS from a 3D-printed master, where the cracks due
to the peeling of PDMS from the interconnected structure of master can be “healed” with a further cure in the oven\textsuperscript{[163]}.

The sacrificial template method has recently been applied to prepare MF channel with junctions and bifurcations. Small organic molecules can either embed in liquid state of PDMS oligomer solution\textsuperscript{[164]} or form the free-standing complex structure before immersion into PDMS\textsuperscript{[128]}. After PDMS solidifies into elastomer upon heating, small molecules can be easily removed by dissolving\textsuperscript{[164]} or evaporation\textsuperscript{[128]}. However, the formation of the template is, up to now, not well controlled compared with lithography, and thus affects the consistency of the resulting PDMS MF channels.

Photoresist reflow is the shape transformation of a positive photoresist due to heating with a temperature above its glass transition temperature ($T_g$). Because of the interfacial tension between the liquid state of the photoresist and the silicon substrate, and the surface tension of the photoresist, the resist prefers to melt into a convex shape. Both semi-circular\textsuperscript{[165]} and circular shape\textsuperscript{[166,167]} of the PDMS MF channels have been prepared by the reflow method, depending on whether a replica is bonded to a planar surface or an identical replica. The combined advantage of reflow feature and soft lithography provides the versatility in preparing cylindrical MF channels. Complex patterns of positive photoresist with junctions and bifurcations were prepared and shown to reflow into semi-circular successfully\textsuperscript{[166,167]}. However, the semi-circularity could not be maintained if the widths of the original rectangular shaped channels are different. Despite the tendency to re-organize into convex shape during reflow, the identical height but various widths of photoresist before reflow restricts the formation of 3D structures. This limitation was overcome in this thesis work, and the details are provided in Chapter 6 of this thesis.

1.3.2.2 Other polymers

In addition to PDMS, polymer materials such as polystyrene (PS), poly(lactic acid) (PLA), poly(DL-lactic-co-glycolide) (PLGA), and poly(glycerol sebacate) (PGS) have been fabricated into a functional endothelialized microvascular network. For example, a simple replication of PGLA structure from PDMS stamp, followed by a thermal bonding process produced a biodegradable microdevice\textsuperscript{[168]}. Another biodegradable polymer, PGS, was prepared with almost the same method, but from a silicon mold instead of a PDMS one\textsuperscript{[169]}. More recent work has upgraded the cross-section of the network from rectangular to
circular. With the aid of an electroplating process, a branched channel network with varying dimensions between generations was prepared\cite{170}. The pattern on the silicon master was transferred to PS substrate via a hard embossing process, rendering an artificial microvascular network with gradual transitions between generation of vessels. PLA network with rectangular cross-sections can be reshaped into circular with an air expansion recipe while heating the material above its glass temperature transition\cite{171}. The single step modification simultaneously transforms the interior topology of bifurcating network with multiple generation of MF channels. In the last two studies above, a confluent seeding of endothelial cells was achieved along the inner surface of the MF channel, suggesting the potential usage of the fabricated channels as blood vessels in tissue engineering. Recently, a protocol named vaporization of sacrificial components (VaSC) was proposed to fabricate 3D interconnected structure in epoxy resin\cite{172,173}. The method involves the preparation of 3D freestanding sacrificial template (PLA) by a commercial 3D printer, the immersion of the PLA template into resin, and the subsequent removal of template by thermal depolymerization and evaporation (over 200 °C) of PLA. However, the quality and smoothness of the final structure depends on the resolution provided by the 3D printer (> 200 μm). The application of 3D printer in building of microvascular network will be discussed later in this section.
Figure 1.3 Artificial microvascular systems prepared from various materials. (A) Computational simulation and (B) microscopic images of four-tumor-cell cluster in transit through a PDMS channel with a circular cross-section. The cluster of cells approach (i), unfold/elongate (ii), travel (iii) and eventually (iv) exit/reform from the constriction. Scale bar = 50 µm. Copyright 2016 National Academy of Sciences. (C) Confocal fluorescent microscopic images of the bilayered architecture consisting of smooth muscle cells (green) as the first layer and endothelial cells (red) as the second layer, seeded on the inner surface of a hollow tubular structure within γ-PGA gel. Scale bar = 100 µm. Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA. (D) A 3D reconstructed confocal image of an endothelial cell lumen with a tubular shape. The lumen was cultured for 48 h and stained for CD31 (green) and nuclei (blue). Scale bar = 100 µm. Copyright 2012 Elsevier Ltd. (E) Fluorescent image of a fully perfused MF network mimicking arteriovenous circulation. Scale bar = 100 µm. Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA. (F) and (G) Proof-of-concept demonstration of evolving MF systems with merged bright-field and fluorescent image of parallel MF channels. (F) Only top channel is perfused with FITC-Dextran (2000 kDa). (G) After laser ablation during the perfusion, fluorescent solution is redirected towards the lower channel. Scale bar = 100 µm. Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA. (H) Schematic of omnidirectional printing of 3D microvascular networks in a hydrogel reservoir. After deposition of fugitive ink (red) in a 3D branching manner, the hydrogel is cross-linked by UV exposure, and a negative pressure is applied to remove the liquefied ink (white branches). Copyright 2011 WILEY-
1.3.3 Hydrogel-based vasculature

In contrast to the polymer (including PDMS) mentioned above, hydrogels are composed of cross-linked hydrophilic polymeric network with high water content. The highly controllable crosslinking density, mesh size, and mechanical properties, along with the efficient mass-transport of oxygen, nutrients and other metabolites facilitates its resemblance with endothelial extracellular matrix (ECM). Typical examples of hydrogels include but are not limited to agarose\cite{174}, collagen\cite{175-177}, poly(γ-glutamic acid) (γ-PGA)\cite{178}, polyethylene glycol (PEG)\cite{179}, and GelMA\cite{180}. Similar to polymer-based vasculature described above, microstructure of hydrogel network can be obtained from a template either below or inside of the hydrogel substrate. Due to the attempt to mimic blood vessels, only few work fabricated MF channels with rectangular cross-section\cite{174}, and a variety of methods have been designed to prepare hydrogel channels with circular cross-section.

Needle-based subtractive molding, involving the gelation of hydrogel around a cylindrical tube and subsequent manual removal of the tube, renders single\cite{175,180} or multiple parallel MF channels\cite{176,178}. Moreover, the inner surface of channels was successfully covered by either single- or multi-layered cells to mimic the barrier function of native blood vessels. For example, needle-based molding followed by a hierarchical cell deposition was applied to prepare bi-layered structures of cells (endothelial cells and smooth muscle cells) along with the inner surface of MF channels made of γ-PGA (Fig. 1.3C)\cite{178}. The analogy of MF channels with blood vessels in terms of barrier function was proved by the restricted permeation of bovine serum albumin (8 nm) through the artificial multilayered blood capillary. Although needle-based method allows easy manipulation of size, length and relative distance of MF channels, it cannot be expanded to form complex networks with junctions or bifurcations which are main geometry features of microvascular system.
In contrast to the tube assistance in the microfabrication, a tubeless protocol was adopted to prepare side-by-side interconnected cylindrical MF channels made of collagen\[177\]. The so-called viscous finger patterning required only a micropipette to fill and aspirate hydrogel solution in multiple steps, in order to leave the solution in the desired positions within a PDMS frame. After gelation and subsequent coverage of endothelial cells in the inner surface of collagen, endothelial-line lumens with circular cross-section were formed (Fig. 1.3D). In addition to test the barrier function, a significant growth of endothelial cells induced by vascular endothelial growth factor (VEGF) was found, indicating the potential adaptability of hydrogel MF channels to model angiogenesis in vitro. Angiogenesis, the sprouting of endothelial cells from existing vessels to form new vessels, is a significant biological process involved in both physiologies and pathologies\[181\]. This process inspired the creation of microchannels via focused nano- or femto-pulsed lasers\[179\]. The laser photo-ablation guarantees the creation of hollow MF channels within hydrogel substrate at any time and location during cell-culture without any post-processing steps. Such techniques can apply to a variety of ECM hydrogels, and proved to be biocompatible with 3D cell culture. A fully perfused PEG hydrogel capillary-bed-mimicry MF network have been successfully produced (Fig. 1.3E). Moreover, evolving MF channels on demand was attained with an additional laser ablation during the perfusion, leading to the redistribution of liquid within MF network (Fig. 1.3F and G). Other techniques to prepare 3D hydrogel MF network employed a strategy similar to the sacrificial template method mentioned in the Section of 1.3.2 “Polymer-based vasculature”, where interconnected hollow gel structures from the removable template of either carbohydrate glass\[182\] or fugitive ink (Fig. 1.3H)\[183\] were reported.

1.3.4 3D printing of vasculature

3D printing, developed in the early 1980s, became publically and scientifically accessible since 2009 with the expiration of a key patent\[184\]. In this printing technology, a designed structure of interest is first digitally sliced into numerous 2D individual layers, and sequentially built by the 3D printer in a layer-by-layer manner. In terms of applied technology, current 3D printing method for polymeric MF channels generally includes four approaches, namely, inkjet 3D printing, fused deposition modelling, stereolithography, and two photon polymerization. Except two photon polymerization (time-consuming and expensive) the resolution of which can reach sub-100 nm, other three methods have difficulty to print features less than
200 µm\textsuperscript{184,185}, and the smoothness of the printed feature is significantly poor compared to the polymeric structure from soft lithography. Another limitation of 3D printing in MF devices is the choice of material. For example, in stereolithography and inkjet printers, the resin must be photo-polymerizable, leaving a gap in biological and mechanical properties between printed scaffold and microvascular system\textsuperscript{186}. So far only one example of 3D printed PDMS structure was reported, however, the transparency of PDMS cannot be maintained due to the inevitable mixing colored photoresist with PDMS\textsuperscript{187}.

Despite these constraints, several attempts have been made to prepare MF channels with optimized conditions. For example, a novel 3D sugar printer extruded melted sugar into PDMS substrate through a nozzle and the sacrificial sugar was removed with boiling water after the PDMS layers were sealed. By controlling the size of the nozzle and applied pressure in extrusion, a minimum MF channel diameter could reach 40 µm\textsuperscript{188}. Sacrificial agarose templates were also adopted to prepare 3D branching network made of photocrosslinkable hydrogels, followed by the vacuum or manual removal of the template\textsuperscript{189} (Fig. 1.3I). The perfusible channels span 150 - 1000 µm, and promote viability and differentiation of cells within hydrogel compared to block hydrogel without microchannels. Another work extruded alginate into gelatin support baths with a 3D printer\textsuperscript{190}. After the printing and alginate gelation, gelatin support was heated at physiological condition and removed. Complex in vivo models (e.g., a coronary arterial tree) can be produced with high structural fidelity (Fig. 1.3J and K). Notably, the resolution of this printing method is still restricted to 200 µm.

To summarize, with the aim of fabricating an artificial microvascular system, hydrogel-based vasculature resembles in vivo blood vessels due to its inherent and dynamically controllable properties. However, there is difficulty controlling growth direction, shape, and dimension of new blood vessels during angiogenesis. Polymeric artificial vasculature, on the other hand, is superior in integrating multiple parameters (e.g., the geometry of bifurcations, control of fluid flow, structural rigidity and long-standing usage) into a single system. In order to mimic the compliance of blood vessels, ultra-soft PDMS needs to be employed to prepare the microfluidic channels. Compared with these two systems, silicon- and glass-based systems have diminished in popularity due to their lower relevance for mimicking in vivo systems in terms of mechanical properties and complex geometries.
1.4 Objectives and thesis content

In this chapter, we have overviewed the current state of research of thromboembolism and fibrinolysis, as well as experimental models of microvasculature. The complicated relationship between the composition, structure, mechanical properties of occlusive blood clots is believed to govern their thrombolytic resistance. Establishing an *in vitro* model to study embolic effects and lysis of occlusive clots would be invaluable in unveiling the underlying mechanism of thromboembolism and fibrinolysis. We propose to use a microfluidics (MFs)-based “double-modeling” platform as the *in vitro* model system. In terms of the “surrogate” clots, MFs can be used to generate spherical hydrogel particles with controlled composition, size, and mechanical properties. The bio-inspired microvasculature is a circular cross-section, PDMS-based microchannel with a constriction. The know-how for systematically fabricating these MF channels with nearly circular cross-sections over multiple generations of sizes is developed in thesis.

*The objective of this thesis is, thus, to establish an in vitro microfluidic model for studies of thrombosis and thromboembolism.* The thesis is organized into the following chapters.

Chapter 2 summarizes the materials and methods used for the preparation of hydrogel particles (microgels) and MF cylindrical channels, as well as the instrumentation and methodologies of data acquisition and analysis.

Chapter 3 describes the design of a pressure-driven system for studies of the behavior of a spherical microgel into a narrow tube (microchannel) with a tapered region. It examines the variation of the microgel position and volume in the tube with applied pressure drop, and also investigates the relaxation of the shape and volume of the microgel released from the constriction to the channel-at-large. The polymer examined in this chapter is the polysaccharide agarose, because agarose gels are excellent models for unentangled polymer gels. This allows the development of a relatively simple mathematical model to carefully understand the deformation of the microgel during its translocation into the constriction. The choice of agarose is also driven by the existing expertise in the group in the fabrication of agarose microgels, and the need to develop the experimental protocols for a simple microgel system that would eventually be applied to fibrin gels.
Chapter 4 expands the results described in Chapter 3, by conducting experimental and theoretical studies of the translocation of microgels with varying dimensions and mechanical properties through the narrow tube with different geometries. The chapter establishes universal principles governing microgel entrance, passage through microchannels and volume reduction in confinement. We also explore the flow of liquid through occlusive microgels.

In Chapter 5, we describe the experiments mimicking thromboembolism and thrombolysis. We use artificial fibrin clots with controlled dimensions, compositions, structures and mechanical properties, to study their pressure-induced insertion into a narrow microchannel with circular cross-section. Convection-enhanced delivery of thrombolytic drug is investigated for occlusive fibrin microgels.

Chapter 6 introduces the fabrication of bifurcating multi-generation microchannels with nearly circular cross-section. Redistribution of liquid within microchannel network is induced by their selective blockage with fibrin microgels, with the aim to simulate blood flow abnormalities in pulmonary embolism.

Chapter 7 summarizes the results from our experimental and analytical studies, and provides recommendations for future research.
References


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Chapter 2
Materials and methods

2 Materials and methods

2.1 Preparation of microfluidic devices in poly(dimethylsiloxane) (PDMS)

2.1.1 Materials

Trichloro(1H,1H,2H,2H-perfluorooctyl)silane (97%), acetone, methanol, isopropyl alcohol, and hexane were purchased from Sigma-Aldrich Canada Co. (Oakville, Ontario). Sylgard 184 silicone elastomer kit which contained poly(dimethylsiloxane) (PDMS) prepolymer and crosslinker was obtained from Dow Corning Corp. (Midland, MI). Silicon wafers were acquired from Wafer World, Inc. (West Palm Beach, FL). SU-8 3000 negative epoxy resists and SU-8 Developer was procured from MicroChem Corp. (Newton, MA). All chemicals were used as received without further purification.

2.1.2 Methods

2.1.2.1 Fabrication of SU-8 masters

Before the master fabrication, a photomask designed in AutoCAD (Autodesk Inc., CA, U.S.A.) was printed in a material of transparency at 10 μm resolution from CAD/Art Services, Inc. (OR, U.S.A.). To prepare masters, silicon wafers were sonicated in a series of chemicals (acetone, methanol, and isopropyl alcohol) and dried with nitrogen gas to render a clean surface. SU-8 3050 was spun-coated on the wafers with the parameters recommended on the MicroChem website\textsuperscript{[1]}, depending on the desired thickness of the microfluidic (MF) channel. In particular, spinning at 1100 rpm for 30 sec was adopted for a photoresist with a thickness of 150 μm. The photoresist-coated wafer was then placed on a hot plate at 95 °C for 15-min “soft bake” to remove a portion of the solvent. After cooling down to room temperature following the “soft bake”, the wafer was then patterned by a mask aligner (MA6, SÜSS Microtec, Garching, Germany) at the flood exposure mode. To obtain 150 μm-thick photoresist, a total UV-exposure energy of 300 mJ/cm².
was applied on the photoresist, with a quartz substrate placed on top of the photomask to ensure a close contact between the mask and the wafer. A subsequent “post bake” procedure (95 ℃ for 5 min) was then employed to remove the remaining solvent, and stabilize the polymerized pattern of the photoresist. The un-crosslinked portion of the photoresist was then removed by the SU-8 developer, and the patterned photoresist-coated wafer was rinsed with isopropyl alcohol and dried with nitrogen gas.

2.1.2.2 Fabrication of microfluidic devices in PDMS

MF channels were prepared using a standard soft lithography[2]. The surface of photoresist master was rendered hydrophobic by incubating at 75 ℃ for 1 h in a sealed desiccator with 0.5 mL of Trichloro(1H,1H,2H,2H-perfluoroocctyl)silane (97%) in it. Unless mentioned otherwise, a 10 to 1 ratio between the PDMS oligomer and curing agent (both from the Sylgard 184 silicone elastomer kit) was used to prepare the mixture, followed by a degassing procedure under vacuum for 15 min to remove the air bubbles trapped inside the mixture. The degassed PDMS mixture was then poured on the silicon master in a Petri dish and incubated in an oven at 73 ℃ for 4 h for a complete cure. The featured PDMS layer was then peeled off from the master and sealed to a planar PDMS layer via air plasma bonding (550 mTorr for 90 sec) and an overnight incubation (115 ℃) strengthened the bonding and rendered the PDMS hydrophobic.

2.1.2.3 Modification of cross-section of PDMS MF channels

For the results report in Chapters 3 and 4, the rectangular cross-section of the PDMS channels in was modified to a circular one by using a nitrogen-gas-template modification process, following the method originating from our research group[3]. The microchannel was filled in with a solution of silicone oligomer in hexanes, followed by a stream of nitrogen gas passing along the channel. The microchannel with circular cross-section was finally obtained by polymerization of PDMS and evaporation of hexanes upon heating at 90 ℃ for 30 min, with the pressure of nitrogen gas being constant. By tuning the pressure of N₂ stream and
the mixture ratio between silicone oligomer and hexanes, the shape and area of circular cross-section can be controlled.

2.1.2.4 Characterization of the geometry of modified PDMS MF channels

To characterize the shape and dimension of the modified PDMS MF channels, both sacrificial and non-sacrificial methods were applied. In the sacrificial method, PDMS channels were cut perpendicularly to the longitudinal direction of the channel with a blade, and the exposed PDMS surface from cutting was placed on the stage of an optical microscope (Olympus IX71) equipped with a camera (Olympus U-CAMD3). The images were analyzed using Image Pro Plus software (Media Cybernetics, MD, USA).

Confocal laser scanning microscopy (CLSM) was the non-sacrificial method of obtaining cross-sectional details. In this method, a PDMS MF channel was filled with 0.01 mg/mL of aqueous solution of fluorescein isothiocyanate (FITC)-dextran (MW = 70 kDa), and placed on the stage of an inverted confocal microscope (Nikon, Eclipse, Ti-E, Japan). A 200 μm-depth laser scan (excitation wavelength of 488 nm) was performed at increments of 5 μm in the direction perpendicular to the imaging plane of the microchannel (z-direction). the shape of the microchannel cross-section was obtained via a 3D reconstruction of the z-stack of CLSM images (Nikon NIS Elements software).

2.2 Preparation of microgels

2.2.1 Materials

Surfactant Span-80, mineral oil (viscosity of 30 cp), Tris-buffer silane (TBS), and Tween 20 were purchased from Sigma Aldrich (Canada). Phosphate-buffered silane (PBS pH 7.4 1X) was purchased from Fisher Scientific (ON, Canada). Ultralow gelling temperature agarose (SeaPrep) was obtained from Lonza (Switzerland). All chemicals were used as received without further purification. Perfluoroalkoxy alkane
(PFA) tubing with an inner diameter of 0.5 mm was purchased from IDEX Health & Science (WA, USA). Fused silica capillaries were purchased from Molex Inc. (IL, U.S.A.).

2.2.2 Methods

2.2.2.1 Preparation of agarose microgels using a microfluidic droplet generator

Agarose microgels were prepared using a two-phase MF T-junction approach (Fig. 2.1) using protocols previously followed in our research group\cite{4}. An aqueous solution of agarose with a concentration of 2, 3, 4, or 5 wt % in Phosphate-buffered silane (PBS pH 7.4 1X) was heated at 40, 45, 50, or 55 °C, respectively, for 15 min to melt agarose in PBS solution and lower the solution viscosity. The melted solution was injected into the side channel as the dispersed phase, and emulsified in 4% of Span-80 in mineral oil injected into the main channel as the continuous phase. Note that the temperature of the agarose solution was maintained by a water jacket surrounding the inlet tubing. The size of the emulsified droplets was controlled by the relative flow rate between the two phases injected with two independently controlled syringe pumps (Harvard Apparatus 2000 Syringe Pump, U.S.A.). The microgel diameter was varied from 40 to 120 μm. The generated droplet traveled downstream through the MF channel and the outlet tubing which was encased in a 4 °C cold water jacket and eventually into PBS buffer in 4 °C water bath. The gels were left in the bath for 20 min for a complete gelation. The suspension was then centrifuged at 1000 rpm (Multifuge X1R, Thermo Scientific, U.S.A.) at 4 °C for 5 min, and the separated oil phase was removed, followed by the wash with a PBS buffer and transfer into a PBS buffer.
Figure 2.1 Schematic of the MF device for the generation of agarose microgels ($C_{ag} = 2$ wt.%). Inlet optical microscopy image show agarose droplets forming at the T-junction of the device. The height of the device is 150 μm. Scale bar is 50 μm. Collected agarose droplets are cooled for 20 minutes before the microgels are transferred into aqueous phase.

2.2.2.2 Characterization of shape and dimension of the microgels

The size and shape of the agarose microgels were measured and analyzed using an inverted optical microscope (Olympus BX51) and Image Pro Plus software (Media Cybernetics, Silver Spring, MD). The size distribution was obtained by measuring the diameter of more than 4000 microgels prepared under the same condition. An interval range of 1 μm was chosen to plot the count of microgels as a function of the diameter of the microgel. The shape of microgels was characterized by their roundness defined as,

$$R = 4\pi \times \frac{A}{P^2}$$  \hspace{1cm} (2.1)

where $A$ and $P$ are the projected area and perimeter of the microgel, respectively.

2.2.2.3 Measuring stiffness of microgels by micropipette aspiration

The stiffness of microgels of different compositions was determined using the micropipette aspiration technique. A fused silica capillary (Molex Inc.) with an inner diameter of 76 μm was connected to a manometer using perfluoroalkoxyalkane tubing (IDEX Health & Science). The capillary and tubing were filled with TBS containing 0.02 wt% of surfactant Tween 20. The pressure at the capillary tip was controlled with the manometer. A droplet of the microgel suspension was deposited on the bottom surface of a Petri dish mounted on the stage of an inverted microscope (Olympus CKX 41), and a microgel particle was trapped at the opening of the microcapillary without excess pressure. The pressure drop was increased in a stepwise manner at a rate of 25 Pa/min, and the change in the microgel shape was monitored at each step by a microscope camera (QImaging QIClick). A typical series of captured brightfield images of a microgel at different aspiration pressures is shown in Fig. 2.2.
To obtain a measure of the softness of the gel particle, the negative pressure differential applied on the microgel, $\Delta P$, was related to the aspirated length of the microgel, $(x - x_0)$, by the relationship

$$\Delta P = S \frac{(x - x_0)}{R_p},$$

(2.2)

where $x_0$ and $x$ are the lengths of intrusion of the microgel into the microcapillary at $\Delta P = 0$ and after the finite suction pressure, $\Delta P$, is applied, respectively, and $R_p$ is the inner radius of the microcapillary. The quantity $S$, which is the slope of the plot of $\Delta P$ versus $(x - x_0)/R_p$, is the microgel stiffness or a measure of the degree of deformability of the microgel. While the stiffness $S$ is directly proportional to the Young’s modulus of the material, for incompressible, linear, elastic materials, the microgels studied in this work were compressible and could also lose water, when subjected to a stress. Therefore, we adhered to the description of the slope of $\Delta P$ versus $(x - x_0)/R_p$ as an apparent “stiffness”, as has been done previously\textsuperscript{[5]}.

Figure 2.2 Captured images of a fibrin microgel ($D_0 = 250 \ \mu m$) aspirated into a silica capillary with an inner diameter of 76 $\mu m$. Pressure drop of aspiration is provided by a manometer connecting to the capillary with a PFA tubing, and the negative pressure drop is shown in respective images. Scale bar is 100 $\mu m$ and applies to all images.
2.3 Microfluidic setup to study the motion of a microgel through a PDMS channel

2.3.1 Materials

Perfluoroalkoxy alkane (PFA) tubing with an inner diameter of 0.5 mm, three-way connectors, and MF adapters with luer lock syringe connectors were purchased from IDEX Health & Science (WA, USA). BD syringes (60 mL and 5 mL) were purchased from Fisher Scientific (ON, Canada).

2.3.2 Methods

2.3.2.1 Entrance studies

A typical experiment consisted of the monitoring of three stages of the passage of a microgel into the constriction: trapping in the tapered region, transit in the tapered region/partial entry into the constriction, and complete entry into the constriction, all videos recorded with the high-speed camera (Casio EX-F1). The various sections of the MF channel are labeled in Fig. 2.3A. For the MF channels used in Chapters 3 and 4, \(d = 110 \pm 5 \text{ μm}, \ d_c = 42 \pm 2 \text{ μm}, \ \alpha = 15, 30 \text{ or } 45^\circ\). For the MF channels used in Chapter 5, \(d = 193 \pm 5 \text{ μm}, \ d_c = 65 \pm 3 \text{ μm}, \ \alpha = 15^\circ\), and there is an even wider channel-at-large (width of 315 μm and height of 239 μm) connecting the inlet (outlet) and the channel portion with the diameter of \(d\). The details of fabrication of the second type of MF channel will be described in Chapter 5. Once a microgel was trapped in tapered section, the applied pressure difference, \(\Delta P\), was changed by incrementally raising and maintaining the height of upstream reservoir for a period of time (\(\Delta t\)), until the microgel no longer changed its shape and position. For the systems in Chapters 3 and 4, a \(\Delta t\) of 30 sec was found to be sufficient to reach equilibration for all applied pressure drops, ensuring that we studied the static, steady-state response of the microgel for each imposed pressure drop. For the system in Chapter 5, the value of \(\Delta t\) was determined by a MATLAB program (The MathWorks, Inc., MA, U.S.A.) whereby a live monitor of the position was measured and reported, and the value of \(\Delta t\) ranged between 5 to 10 min.
The position of the microgel was quantified by analyzing the experimental video of the entry process in a MATLAB program, that detected the positions of the back edge \( X_b \) and front edge \( X_f \) of the microgel as a function of time. From these values, the position of the microgel was approximated as \( X_c = (X_b + X_f)/2 \).

For the system in Chapter 3, the value of microgel position is with respect to the center of the constriction along \( x \)-axis, while for systems in Chapters 4 and 5, the value is with respect to the starting point of the constriction along \( x \)-axis. A stepwise increase in \( \Delta P \) eventually pushed the microgel completely through the constriction, and such pressure drop was recorded as a critical pressure drop \( (\Delta P_{\text{crit}}) \) in Chapter 3, and as a translocation pressure \( (\Delta P_{\text{max}} \text{ in Chapter 4; } \Delta P_{\text{tr}} \text{ in Chapter 5}) \) in Chapters 4 and 5. By using the same video, the microgel volume was also calculated based on the position of microgel edges within the MF channels with known dimensions characterized with the method mentioned in the Section of 2.1.2.4 “Characterization of the geometry of modified PDMS MF channels”. The method to calculate the microgel volume is explained in Fig. 2.4.

After complete microgel entrance into the constriction, the downstream reservoir was quickly elevated to balance the water level in two reservoirs, and thus \( \Delta P = 0 \). The microgel maintained its position in the constriction and acquired an equilibrium volume, \( V \). Following the same method in Fig. 2.4, both the equilibrium length and volume of the microgel can be calculated.
Figure 2.3 Schematics of the pressure-driven microfluidic system to study entrance and relaxation behavior of microgels within a microfluidic channel with a constriction. (A) Schematic of MF channel with a circular cross-section. $d$, $d_c$, and $\alpha$ are the diameter of the channel-at-large, the diameter of the constriction, and the entrance angle from the channel-at-large to the constriction along $x$-axis, respectively. The tapered section connects the channel-at-large and the constriction. (B) Microfluidic setup for studies of entrance behavior of the microgel into the constriction. A pressure drop was imposed by varying the difference of height of water ($\Delta H$) in upstream and downstream reservoirs. Microgels were introduced into the MF device by a syringe (microgel feeder) connected to the upstream reservoir using a three-way valve. A high-speed camera was focused on the tapered zone from the channel-at-large to the constriction, to record the entrance of the microgel into the constriction.
Figure 2.4 Calculation of volume change of the microgel during its progression into the constriction based on the captured images. In state 1, the microgel consists of three parts (two spherical caps and a circular truncated cone); in state 2, a microgel consists of four parts (two spherical caps, a circular truncated cone, and a cylinder).

\[
\text{State 1: } V = \frac{\pi}{6} h_1 (3r_1^2 + h_1^2) + \frac{\pi}{3} H (r_1^2 + r_1 r_3 + r_3^2) + \frac{\pi}{6} h_3 (3r_3^2 + h_3^2) \\
\text{State 2: } V = \frac{\pi}{6} h_1 (3r_1^2 + h_1^2) + \frac{\pi}{3} H (r_1^2 + \frac{1}{2} r_1 d + \frac{1}{4} d^2) + \frac{\pi}{4} d^2 L_3 + \frac{\pi}{6} h_4 (\frac{3}{4} d^2 + h_4^2)
\]

2.3.2.2 Relaxation studies

After confining the microgel in the constriction for a constant period of time (20 min for system in Chapter 3; 1 h for system in Chapter 5), we released the particle into the channel-at-large (width of 300 \(\mu\)m and height of 150 \(\mu\)m in Chapter 3; width of 315 \(\mu\)m and height of 239 \(\mu\)m in Chapter 5), and monitored the shape relaxation and area relaxation of the microgel with time. The time of recovery was counted from the instant the microgel entered the channel-at-large after being released from the constriction. Captured images (from the high-speed video in system of Chapter 3; from optical microscopic images in system of Chapter 5) displayed its transient shape and area, compared to those of an unperturbed microgel. Both the area and the shape of the microgel were measured by a MATLAB program, and the shape is defined as the ratio between the long and short axes of the microgel observed in the images. In Chapter 5, in addition to area and shape, the relaxation of birefringence, associated with the fiber alignment within the microgel, was recorded using polarized optical microscopy (POM) images from an upright microscope (Nikon BX51) equipped with a digital camera (Media Cybernetics, MD, USA). Since polarized and bright field microscopy images could not be obtained at the same time, an alternate capture of the microgel with polarized and bright field microscopy was employed. The birefringence was represented by the grey scale.
of POM brightness of microgel (profile of which obtained from the respective bright field microscopic images) measured by a MATLAB program.
References


Chapter 3
The motion of microgel in an axisymmetric constriction with a tapered entrance

3 The motion of a microgel in an axisymmetric constriction with a tapered entrance

3.1 Introduction

The flow of soft objects in confined geometries has been a subject of extensive investigation, particularly due to its significance in the biological and biomedical fields\cite{1-4}. For example, in tissue engineering, hydrogels experience large shape deformations in needles or catheters, and have to maintain sufficient integrity and strength for subsequent recovery\cite{2}. In microcirculation, red blood cells can, quite remarkably, pass easily through capillaries with diameters smaller than cell dimensions\cite{3}. Neutrophils (6 - 8 μm), on the other hand, are less deformable, and show much higher transit times for passage through the pulmonary capillary bed, which is composed of capillaries much smaller than the neutrophil. These large transit times, that range from a few seconds to 20 min or greater, create a reservoir of neutrophils in the lungs that is believed to be employed by the immune system to fight infection\cite{4}. Diseased blood cells can exhibit reduced ability to deform, which may lead to compromised blood flow through the microvascular system\cite{5-7}, and trigger pathophysiological effects such as anemia\cite{8} and sepsis\cite{9}.

Although the importance of understanding dynamics of the motion of soft particles through geometrical confinements has never been underestimated, its mechanism is complicated, and experimental systems providing simple qualitative and quantitative guiding principles are required. This need has led to studies based on different types of synthetic soft objects, namely capsules\cite{10}, microgel particles (microgels)\cite{11}, and vesicles\cite{12}. Among these, the microgel, comprised of a water-swollen polymeric network, represents a promising model system. This is because of the straightforward synthesis of microgels with controllable sizes, well-defined shapes and a homogeneous morphology\cite{13}. Microgels can be prepared to be highly deformable, allowing them to be squeezed into and transported in pores that are as much as ten-fold smaller in size, thus acting as a model particle for renal filtration\cite{14}. Such conformational flexibility is also a highly favorable characteristic in the field of drug delivery. Indeed, microgels can be designed to release preloaded drugs in response to external stimuli\cite{15}, being well-preserved as drug carriers when transported through
thin vessels and capillaries[16]. Moreover, the flow behavior of hydrogels through the microvascular system is similar to real blood cells[17], indicating the potential of using hydrogels to simulate and understand deformation of blood cells under passive forcing (as opposed to active forcing, such as occurring in actin depolymerization and pseudopod formation[18]).

Soft particles have been tested in different model environments with micrometer-sized dimensions, such as microfluidic (MF) channels[5–7,19,20] and capillary tubes[21–23]. While rigid MF channels (e.g. glass or silica microcapillaries) have been used frequently to monitor the flow and deformation of soft objects[21–28], these channels suffer from several drawbacks, a key challenge being that it is difficult and expensive to fabricate complex channel networks of tunable dimensions in such substrates. This challenge has been overcome by the development of soft lithography, whereby intricate networks of microchannels with controlled geometries can be fabricated in poly(dimethylsiloxane) (PDMS)[29,30]. There have been several experimental studies of the flow of soft particles through PDMS channels[5,19,29,31,32]. Unfortunately, these studies had an important drawback – the inherent rectangular nature of the cross-section of the PDMS channel[33]. When a soft particle acquires a steady position in a tapered, rectangular constriction, liquid may flow through the four corners of the cross-section, resulting in reduced pressure build-up across the particle. This residual flow can also bring more particles to the constriction, which interferes with the characterization of the flow behavior of a single particle. In addition, correlation between theory with experimental results is complicated, due to the difficulty in the determination of the cross-sectional shape of the arrested particle using conventional brightfield/darkfield microscopy[34].

In the current chapter, our objective was to establish fundamental understanding of the mechanisms of entrance and exit of a microgel through an axisymmetric constriction with a tapered entrance. Such confinements were created using conventional soft lithography, followed conversion of the rectangular cross-section to a circular one[35]. While prior studies have focused on studying the motion of soft particles through constrictions under constant flow rate conditions[11,17,20,36], our studies were performed at constant pressure difference or pressure drop across the microgel. This type of experiments enabled us to establish the relationship between the microgel position in the constriction and applied hydraulic pressure drop along the microchannel. We found the influence of microgel size on the critical pressure drop, $\Delta P_{\text{crit}}$ - the minimum pressure required to force the microgel into the constriction. During the entrance of the microgel into the constriction, significant changes in both its shape and volume were observed. A simple theoretical
model employing a static force balance over the microgel was developed, which agreed well with the experimental data. We also performed studies of the relaxation of the shape and volume of a microgel after its exit from the constriction, and discovered that the time scales of shape and volume relaxation of a deformed and deflated microgel were different. We believe this is a systematic effort to provide fundamental, step-by-step understanding of the entrance of a microgel into an axisymmetric constriction, and the relaxation of the microgel to its initial state upon its release from the constriction.

### 3.2 Design of microgel flow experiments

In this chapter, the PDMS MF channel with a circular cross-section was obtained following the nitrogen-gas-template method described in detail in the Section 2.1 “Preparation of Microfluidic devices in PDMS”. In particular, the diameter of the channel-at-large is \( d = 110 \pm 5 \, \mu\text{m} \), the diameter of the constriction is \( d_c = 42 \pm 2 \, \mu\text{m} \), and the entrance angle (\( \alpha \)) from the channel-at-large to the constriction along \( x \)-axis is 15°, respectively, as shown in Fig. 3.1. The agarose microgels (2 wt%) were prepared in the size range from 40 to 120 \( \mu\text{m} \), according to the method described in detail in the Section 2.2.2.1 “Preparation of agarose microgels using a microfluidic droplet generator”. The experimental setup to study the microgel flow has been described in detail in the Section 2.3 “Microfluidic setup to study flow of a microgel through a PDMS channel”. Briefly, the flow of an individual microgel within the PDMS MF channel was controlled by varying the difference of water level in upstream and downstream reservoirs, and the motion of the microgel was recorded by a high-speed camera and analyzed by a MATLAB program.
Figure 3.1 Schematic of MF channel with a circular cross-section. $d$, $d_c$, and $\alpha$ are the diameter of the channel-at-large ($d = 110 \pm 5 \mu m$), the diameter of the constriction ($d_c = 42 \pm 2 \mu m$), and the entrance angle from the channel-at-large to the constriction along $x$-axis ($\alpha = 15^\circ$), respectively. The length of tapered section connecting the channel-at-large and the constriction is 120 $\mu m$. The abscissa $x = 0$ denotes the center of the constriction.

3.3 Results and discussion

3.3.1 Preparation of agarose microgels and PDMS MF channel

The prepared 2 wt% agarose microgels had a mean diameter of 99 $\mu m$, with a polydispersity (defined as the standard deviation in the dimensions of the microgels divided by the mean diameter) of 4.2%, as shown in Fig. 3.2. The microgels had a well-defined round shape with roundness close to 1, as defined in section 2.2.2.2 “Characterization of shape and dimension of the microgels”.

Figure 3.2 Optical microscopy images (A) and size distribution (B) of agarose microgels transferred in water. $C_{ag} = 2$ wt.%. To obtain size distribution, 4478 microgels were counted. Scale bar is 50 $\mu m$.

The PDMS MF channel with a circular cross-section was characterized by the sacrificial method, and the cross-sectional views of both channel-at-large and constriction was shown in Fig. 3.3.
Figure 3.3 Bright field images of the cross-sectional area of the microchannels (A, C) and constrictions (B, D) produced at $C_{PDMS} = 50\%$. $P_{N2}$ is of 6 psi (A, B) and 12 psi (C, D). Scale bar is 100 μm and applies to all figures.

3.3.2 Entrance studies of the microgel to the constriction

A typical experiment consisted of the monitoring of three stages of the passage of a microgel into the constriction: trapping in the tapered region, transit in the tapered region/partial entry into the constriction, and complete entry into the constriction, all videos recorded with the high-speed camera. The various sections of the MF channel are labeled in Fig. 3.1. Once a microgel was trapped in tapered section, no gaps existed between the microgel and the walls of the microchannel. The applied pressure difference, $\Delta P$, was changed by incrementally raising and maintaining the height of upstream reservoir for a fixed time, $\Delta t$. At each pressure drop, we waited until the microgel no longer changed its shape and position. For the system studied, a $\Delta t$ of 30 sec or greater was found to be sufficient to reach equilibration for all applied pressure
drops (Fig. 3.5A), ensuring that we studied the static, steady-state response of the microgel for each imposed pressure drop.

Fig. 3.4A shows the variations in pressure drop across the microgel (blue curve, left ordinate), and the position of the microgel center $X_c$ (red curve, right ordinate), plotted as functions of time, for $\Delta t = 60$ sec and $D_0 = 94 \mu$m. The position of the microgel was quantified by analyzing the experimental video of the entry process in a MATLAB program, that detected the positions of the back edge ($x = X_b$) and front edge ($x = X_f$) of the microgel as a function of time. From these values, the position of the microgel was approximated as $X_c = (X_b + X_f)/2$. Each incremental step of $\Delta P$ led to movement of the microgel further out of the tapered section and into the constriction. Note that upon a step increase in $\Delta P$, the position of the particle did not exhibit an instantaneous change; rather, it required time to reach its steady-state position and shape (Fig. 3.4A). A more detailed progression behavior of front edge and back edge of the microgel with time was shown in Fig. 3.6. When the value of $\Delta P$ exceeded a critical pressure drop, $\Delta P_{\text{crit}}$, the microgel did not achieve a steady-state position. Instead, it exited the tapered section, completely entered the constriction and traversed it, all in one continuous motion.

The dependence of the microgel position on the pressure drop is shown in Fig. 3.4B, where each imposed pressure drop corresponds to a steady-state position of the microgel in the constriction. The curves represent an average of the data for ten microgels with diameters of $99 \pm 1.0 \mu$m, as may be verified from Fig. 3.5B.

**Figure 3.4** The effect of a pressure drop across the microgel on the position of the microgel center in the microchannel. (A) With the increase of pressure drop (blue line), the position of the microgel center (red line) moved downstream toward the microchannel entrance. The numbers 1 to 8 indicate the eight steady-state positions of the microgel caused by eight step increases in the pressure drop, each of magnitude
250 Pa. Point 9 corresponded to a pressure drop greater than the critical pressure drop for pushing the microgel through the constriction. (B) Relationship between pressure drop and the position of microgel center in (A). For both subfigures, $\Delta t = 60$ sec and $D_0 = 94$ μm.

Figure 3.5 Reproducibility of effect of (A) maintaining time ($\Delta t$) and (B) pressure drop on the position of multiple microgels ($D_0 = 99 \pm 1.0$ μm). Four gels for (A) and ten gels for (B). (A) Four maintaining time was chosen (from 0.5 minute to 3 minute), and the position of the microgels is found to be always within the average position obtained (B).
Figure 3.6 Effect of the pressure drop across the microgel on the position of the front edge (black), center (red), and back edge (blue) of the microgel in the device. (A) The progression of respective positions with time. $\Delta t = 60$ sec, $D_0 = 94$ μm. (B) - (D) shows the shape of the microgel at the starting position (138 sec), at the intermediate position (144 sec) and at the equilibrium position (156 sec) of the microgel in the microchannel when the pressure drop was increased from 110 Pa to 350 Pa at 138 sec. The two red lines indicate the back edge at 138 sec and the front edge at 156 sec, respectively.

We then examined the effect of microgel size on the critical pressure drop, $\Delta P_{\text{crit}}$. In these experiments, the pressure drop was increased in a step-wise manner, with a step size of 50 Pa. Therefore, the true $\Delta P_{\text{crit}}$ is expected to be overestimated by 50 Pa, at the most. Fig. 3.7 shows the dependence of $\Delta P_{\text{crit}}$ on microgel size for in the range 56 μm $< D_0 < 107$ μm for the channel geometry shown in Fig. 3.1. With increasing microgel diameter, a greater pressure drop was required to achieve complete microgel passage into the constriction, caused by the stronger shape and volume change experienced by larger particles during the passage.

![Figure 3.7 Effect of microgel diameter ($D_0$) on the critical pressure drop ($\Delta P_{\text{crit}}$).](image)

Microgels with sizes over a range (56 $< D_0 < 107$ μm) were used for the same MF channel. The diameters of the channel-at-large and the constriction were 109 μm and 42 μm, respectively. For all experiments in the figure, $\Delta t = 60$ sec.
Figures 3.8A and B, show representative microgel shapes in the channel-at-large and within the constriction, respectively, for a microgel that was forced completely into the constriction by employing a $\Delta P > \Delta P_{\text{crit}}$. Quantitative comparison of these shapes suggested that there is a significant reduction in volume of the microgel upon its entry into the constriction, which was ascribed to the loss of water by the microgel particle. The volume loss experienced by the microgels is conveyed in Fig. 3.8C, which presents the volume of the microgel relative to its initial volume, $V/V_0$, plotted as a function of $\Delta P$. The method of calculation of the microgel volume was explained in Fig. 2.4 in Chapter 2. The relative microgel volume decreases monotonically as the microgel enters the constriction. The loss of volume by the microgels indicates that they are not incompressible, and have a finite bulk elastic modulus $K$, as also recorded previously using osmotic deswelling$^{[37]}$.

![Figure 3.8 Volume reduction of the microgel with the progression into the constriction.](image)

(A) A microgel confined by the constriction ($d_c = 42 \mu m$), with the volume of $1.18 \times 10^5 \mu m^3$, approximately, from its resting spherical shape (B) in the channel-at-large ($D_0 = 77 \mu m$), with an approximate volume of $2.39 \times 10^5 \mu m^3$. The Scale bar is 50 $\mu m$. (C) Detailed values of microgel volume (blue dots) with its progression into the constriction (red dots) induced by pressure drop.

There have been several prior works elucidating the pressure-driven motion of soft particles in confined geometries$^{[38-48]}$. However, to our knowledge, there has been only one publication$^{[49]}$ that examines the motion of fully elastic particles (as opposed to elastic capsules or vesicles) with a finite bulk modulus under confined conditions. We have employed a semi-analytical model based on a slightly modified version of
this recent work\[49\] for predicting the position and the volume change of the microgel subjected to a static pressure drop, \(\Delta P_{\text{stat}}\). The details of the model are provided in the Appendix. In essence, the model derives the deformations of the microgel in the axial and radial directions, and in turn, the appropriate elastic stresses that balance the applied pressure difference \(\Delta P_{\text{stat}}\) and the repulsion from the wall, \(P_w\) (Fig. 3.9).

![Figure 3.9 Schematic of microgel deformation.](image)

The variables \(r\) and \(x\) are the radial and axial co-ordinates. The microgel shape comprises three parts: two spherical caps, \(S_l\) and \(S_R\), on the upstream and downstream sides of the microgel, and a region \(S_C\) that contacts the wall. \(\Delta P_{\text{stat}}\) is the pressure difference, \(P_L-P_R\), across the microgel. \(P_w\) is a repulsive pressure resulting from the reaction force from the wall. It balances the differences in the forces due to \(P_L\) and \(P_R\) to maintain the microgel in a steady-state position.

In Fig. 3.10A, we show the prediction of the position of the microgel, \(X_c\), as a function of \(\Delta P_{\text{stat}}\) from the theory. The Young’s modulus of agarose microgels has been recently measured to be 2.9 kPa\[50\]. We have taken the Poisson’s ratio of the bulk microgel to be 0.38, obtained by fitting the microgel position (Fig. 3.10A) and the relative volume (Fig. 3.10B) predictions to the experimental data presented in Fig. 3.8. This yields the bulk and shear moduli as \(K = 4.2\) kPa and \(G = 1.0\) kPa respectively. We have also indicated the values of \(\Delta P_{\text{stat}}\) corresponding to the microgel shapes in Figs. 3.10A and B. In the case shown in Fig. 3.10C(i), the value of \(\Delta P_{\text{stat}}\) is identically zero. In this position, the microgel just contacts the microchannel walls, and undergoes no deformation from its initial spherical shape. Beyond this position, e.g. Fig. 3.10C (ii), the contact area between the microgel and the microchannel wall is increased, leading to a \(\Delta P_{\text{stat}}\) increase. For positions in which the microgel is progressively moving into the constriction [Fig. 3.10C (iii)-(v)], the degree of tapered experienced by the microgel reduces, thereby decreasing \(\Delta P_{\text{stat}}\). Ultimately, in case Fig. 3.10C(vi), when the microgel is completely within the constriction, \(\Delta P_{\text{stat}}\) is zero, because it adopts a fore-aft axisymmetric shape, due to the absence of taper in this region.
In Fig 3.10A, we note that $\Delta P_{\text{stat}}$ is always less than or equal to $\Delta P_{\text{crit}}$. Also, the portion of the curve of $X_c$ vs. $\Delta P_{\text{stat}}$ for $X_c > X_{\text{crit}}$ is shown with a dotted line. This is because the static steady states corresponding to these positions are unstable, and inaccessible in our experiments. Consider a microgel trapped in a static steady state under an applied pressure difference equal to the critical value, that is, $\Delta P = \Delta P_{\text{stat}}(X_{\text{crit}}) = \Delta P_{\text{crit}}$. If the microgel experiences a small perturbation from this position, so as to be pushed further towards the constriction, i.e., if $X_c = X_{\text{crit}} + \delta$, with $\delta > 0$, the pressure difference required to sustain the microgel in a static steady state in this new position is lower than the applied pressure difference $\Delta P_{\text{crit}}$ being imposed on the microgel $|\Delta P_{\text{stat}}(X_{\text{crit}} + \delta) < \Delta P_{\text{crit}}|$ (Fig. 3.10A). This pressure imbalance, $\Delta P - \Delta P_{\text{stat}}(X_{\text{crit}} + \delta)$ means that the microgel will be displaced further into the constriction (Fig. 3.1), enhancing the difference $\Delta P - \Delta P_{\text{stat}}$. Ultimately, the imbalance will lead to the microgel entering the constriction completely (a similar argument may be employed to show why the steady states for $X_c < X_{\text{crit}}$ are stable).

Fig. 3.10B shows the theoretically-predicted and experimentally measured (reproduced from Fig. 3.8C) volumes of the microgel normalized by its initial volume, plotted as a function of the static pressure drop applied across the microgel. The agreement between experiment and theory is excellent, with the maximum error being less than 10%. The volume of the microgel decreases as it is forced into the tapered region of the constriction under the influence of the applied pressure drop, and this occurs up to the critical pressure drop. Beyond this position, the static pressure drop across the microgel decreases, leading to partial recovery of the volume of the microgel. As in the case of the variation of $X_c$ vs. $\Delta P_{\text{stat}}$ in Fig 3.10A, the lower half of the curve is not accessible by experiment, and is shown with a dotted line.

It is important to mention here that the calculations of $\Delta P_{\text{stat}}$ and $V/V_0$ are based on a linear elastic model with constant elastic moduli. In reality, the loss of water leads to an increase in the concentration of the polymer in the microgel, and hence, to higher values of the elastic moduli. The dependence of the moduli on the degree of deflation of the microgel was not considered in our calculation. Should this effect be taken into account, we anticipate values of the Poisson’s ratio less than 0.4. In addition, we have ignored friction between the microgel and microchannel.
Figure 3.10 Theoretical prediction of variation of center position of microgel and microgel volume as a function of pressure drop. (A) The variation of the position of the center of the microgel, \( X_c \), with the static pressure drop, \( \Delta P_{stat} \), applied across it. (B) The normalized volume of the microgel as functions of the applied pressure difference. (C) The static steady shapes for the microgel in the constriction for the positions (i) through (vi) shown in (A). For all the calculations, we used \( D_0 = 93 \, \mu m \), and a Young’s modulus of \( E = 2.9 \, kPa \). The Poisson’s ratio that fits the data best was found to be 0.38, implying bulk and shear moduli of \( K = 4.2 \, kPa \) and \( G = 1.0 \, kPa \) respectively. The circles in (A) and (B) are reproductions of the experimental data in Fig. 3.4B and Fig. 3.8C, respectively.

3.3.3 Memory effects in microgel motion

To understand the time scales for the significant changes in shape and volume of the microgel during its entrance into the constriction, we performed two types of experiments. In the first type, we examined the microgel shape upon repetitive entrance and exit from the constriction. Four cycles of experiments were implemented, with each cycle comprising three steps, a, b and c, as shown in Fig. 3.11A. In step a, we applied a constant pressure drop \( \Delta P \) for 10 min to maintain the microgel at the entrance of the constriction. In step b, we confined the microgel in the constriction for 5 min by maintaining zero \( \Delta P \), so that the microgel would remain stationary. In step c, after the confinement, we reduced \( \Delta P \) and released the microgel back to the channel-at-large for a period of recovery of \( \tau \) seconds. The cycle of steps a to c was implemented four times. In each cycle, the same pressure drop (1080 Pa) was applied in step a, and the position of the microgel in the tapered region of the constriction was recorded in this step. To go from step a to step b, the outlet reservoir was first lowered to push the microgel into the constriction, and then brought back up to ensure that \( \Delta P \) was zero. The waiting period in step b provided ‘memory’ of shape deformation and volume shrinkage to the microgel, and step c was used to ‘erase’ such ‘memory’ of the microgel. We assumed that for sufficient recovery time in step c, the microgel would reach the same position in step a in every cycle. If not, the position in the second and higher cycles would be deeper in the constriction relative to the first cycles.

Fig. 3.11B shows the position of the microgel in the constriction for recovery times (\( \tau \)) varying from 10 sec to 30 min. For \( \tau \geq 60 \) sec, the position of the microgel in step a in every cycle was constant. For shorter recovery times, e.g. \( \tau = 10 \) sec, or \( \tau = 30 \) sec, the microgel moved deeper into the tapered region in the second cycle. Remarkably, the steady position of the microgel in step a increased monotonically in subsequent cycles, suggesting that step c was not ‘erasing’ the shape deformation memory completely. This
effect on the motion of the microgel was most noticeable in $\tau = 10$ sec study, where the microgel completely entered and traversed the constriction in the fourth cycle, and therefore, no acquisition of its position was possible in that cycle.

These results demonstrate that incomplete restoration of a microgel to its equilibrium shape can lead to an easier passage of the microgel through the same confined geometry under the same pressure drop. Thus, the finite time of relaxation can allow easier passage of a deformed microgel through a constriction. This observation should have relevance in the pulmonary transit of leukocytes, where cells need to navigate through 50 to 100 successively-aligned narrow segments\textsuperscript{[51]} and the transit time varies significantly from one segment to another in experiments\textsuperscript{[52]}. A similar phenomenon was recently reported recently\textsuperscript{[19]} for leukocytes forced to travel through successive narrow segments. In this study, the flow rates and the intersegment spacing were chosen such that the cells did not have sufficient time to recover to their original shapes. Consequently, the cells were found to cross the first constriction in about 25 sec, but in only 0.5 sec for all subsequent constrictions.
3.3.4 Relaxation behavior of a microgel released from the constriction

For a more detailed understanding of the relaxation of microgels, we examined the relaxation of a microgel released from a constriction. After confining a microgel ($D_0 = 86 \text{ μm}$) inside the constriction ($d_c = 45 \text{ μm}$) for 20 min, we released the particle into the channel-at-large (width = 300 μm, height = 130 μm), respectively, and monitored the shape relaxation and area relaxation of the microgel (Fig. 3.12A-D). Note that since the dimensions of the channel-at-large are larger than the microgel size, the relaxation dynamics is not influenced by confinement effects, and the rate of area relaxation can be viewed as a measure of the...
rate of volume relaxation of the microgel. The time of recovery was counted from the instant the microgel entered the channel-at-large after being released from the constriction (Fig. 3.12A). Captured images from the video (Fig. 3.12B-D) displayed its transient shape and area, compared to those of an unperturbed microgel (red dotted circle). Right after it was released from the constriction, the microgel exhibited a non-spherical shape and a smaller area than in the unperturbed state (Fig. 3.12B). After 1.3 sec of recovery, the deviation from the spherical shape almost disappeared, but the area still did not recover to its original value (Fig. 3.12C). A full recovery both in shape and area was observed 20 sec after microgel release from the constriction (Fig. 3.12D). These results indicated that the restoration of the shape was faster than that of the area.

To further understand the difference between the two relaxation processes, we studied their kinetics quantitatively by monitoring the area (Fig. 3.12E) and the aspect ratio (Fig. 3.12F) of the microgel. The aspect ratio is defined as the ratio between the long and short axes of the microgel observed in the images. An exponentially-decaying model, characterized by a single decay time constant, \( t_c \), was adopted to fit both relaxation processes\(^{[53]} \) as \( F(t) = F_\infty - (F_0 - F_\infty) \exp(-t/t_c) \). Here, \( F \) can be the area or the aspect ratio, \( F_0 \) and \( F_\infty \) are the initial and asymptotic (\( t \to \infty \)) values of \( F \). The time constant of aspect ratio relaxation of the microgel was 0.42 sec (Fig. 3.12E), and that of area relaxation was 1.47 sec (Fig. 3.12F), thus indicating longer relaxation time for the microgel area (or volume) relative to the aspect ratio (shape). To the best of our knowledge, these two relaxations have never been simultaneously recorded.

The time scales for the two relaxation processes are shown in Fig. 3.13 for microgels of different sizes. While volume relaxation being slower than shape relaxation is maintained for all microgels, additional trends were observed. According to Fig. 3.13, the time scale for area relaxation increased with the diameter of the microgels, because larger microgels undergo greater water loss and shape deformation upon confinement. Also, the time scale for volume relaxation appears to be more sensitive to the microgel diameter, as compared to the time scale for shape relaxation.

We noted that this relaxation occurs in a special way. The microgel relaxes to a sphere of diameter smaller than the initial diameter, followed by the swelling of the microgel back to its original diameter in a series of spheres of successively increasing radii. The two processes are not sequential, i.e., swelling also occurs during the relaxation of the non-sphericity, but the spherical shape is regained before the microgel regains
its original volume. The understanding of these effects needs further investigation, and we are working on a mechanical model of the microgel to explain these observations.

**Figure 3.12 Relaxation behavior of a microgel from the constriction.** (A-D) Different stages of the microgel released back into unconfined area, $t = 0$, 0.06, 1.31, and 20 sec for A, B, C, and D stage, respectively. Scale bar is 50 μm and applies to all figures. (E, F) Area relaxation and aspect ratio (long/short-axis ratio) relaxation of the microgel as a function of time, respectively. The width and height of the channel-at-large is 300 μm and 130 μm, respectively. $D_0 = 86$ μm, $d_c = 45$ μm, $\alpha = 15°$. 

### Figure 3.12

- **A**
  - Area: 5376 μm²
  - Aspect Ratio: 1.09

- **B**
  - Area: 5800 μm²
  - Aspect Ratio: 1.01

- **C**
  - Area: 6102 μm²
  - Aspect Ratio: 1.01

- **D**
  - Scale bar is 50 μm and applies to all figures

- **E**
  - Area relaxation over time

- **F**
  - Aspect ratio over time
Figure 3.13 The variation of the two relaxation times with the diameter of the microgel. Triangles – time scale for area (volume) relaxation, Circles – time scale for aspect ratio (shape) relaxation.

3.4 Conclusions

In this chapter, we examined the entrance of a spherical microgel into an axisymmetric constriction with a tapered entrance, under the influence of a pressure difference imposed across it. As the pressure drop across the microgel was increased, it moved progressively further into the constriction, and when a critical pressure drop was exceeded, the microgel completely entered and moved through the constriction. For the particular constriction geometry employed in this work, the critical pressure drop increased non-linearly with the size of the microgel. A model based on linear elastic theory was used to explain the experimental data.

The critical pressure difference for entrance of a soft particle has significance in the biomedical field, where diseased blood cells may exhibit reduced ability to deform, resulting in severely compromised blood flow or even occlusion through the microvascular system\textsuperscript{[6–7]}. The knowledge of this critical value should be beneficial for estimation of the possibility of partial or complete blockage of the blood vessels, providing guidance in prevention and treatment of relevant diseases.
The microgel underwent significant volume during its entry into the constriction, and this prompted us to examine the relaxation behavior of a deformed, deflated microgel. We noted that if a microgel had already entered a constriction, a lower pressure drop was required to force it again into the same constriction, provided this was done over a time scale shorter than the relaxation time scale of the microgel. A second major result from the relaxation studies was that a deformed microgel can exhibit multiple time scales in the process of relaxing back to its original shape and size. In particular, it was noted that the relaxation of the non-spherical shape occurs faster than the relaxation of the volume, that is, a deformed, deflated microgel reverts first, to a spherical shape, and then the spherical microgel swells back to its original volume. In addition, the resilient morphology of the microgels indicates the fracture strength of agarose gels has not been reached in the pressure-driven microfluidic system. The relaxation behavior of microgels studied here is not only fundamentally important, but also useful in designing microgels for specific applications. For example, the ability of microgels or microcapsules to preserve integrity and regain their unperturbed shape after passage through thin vessels is highly desirable in the design of drug carriers in vivo\cite{16}.

This chapter established a MF platform, and set the foundation for understanding entrance of microgels through a narrow tube. The modeling system reported in this chapter was further implemented in Chapter 4 to study the translocation of microgels with various dimensions and mechanical properties through the narrow tube with different geometries. The polymer examined was the polysaccharide agarose, because agarose gels are excellent models for unentangled polymer gels. This allowed the development of a relatively simple mathematical model to carefully understand the deformation of the microgel during its translocation into the constriction. The choice of agarose was also driven by the existing expertise in the group in the fabrication of agarose microgels, and the need to develop the experimental protocols that would eventually be applied to fibrin gels as described in Chapter 5.
References

Chapter 4
Universal behavior of hydrogels confined to narrow capillaries

4 Universal behavior of hydrogels confined to narrow capillaries

4.1 Introduction

Properties of geometrically constrained synthetic and biological soft matter govern a large number of physical phenomena, including flow of complex liquids through narrow pores, confinement-induced phase transitions and shape transformations, wetting, adhesion and friction. In particular, flow of discrete soft matter objects through narrow quasi-one-dimensional capillaries or tubes is of great importance for industrial applications, e.g., in enhanced oil recovery\(^{1,2}\) and in biological and biomedical systems\(^{3–19}\). For example, blood clots can cause vascular occlusions and result in myocardial infarctions\(^{8}\), stroke\(^{9}\) and pulmonary embolism\(^{10,11}\). On the other hand, embolization of blood capillaries with soft polymer particles is utilized to reduce blood supply to tumor sites or treat endovascular aneurysm\(^{13–16}\). Furthermore, small nanometer-size conformable particles of biological matter can be cleared from the body by renal (glomerular) filtration through pores defined by narrow endothelial gaps\(^{17}\). Other exemplary systems include needle- or catheter-assisted injection of hydrogels for drug delivery\(^{18}\) and tissue engineering\(^{19}\).

While the systems listed above may belong to different classes of soft matter with a broad range of chemical and biophysical properties, upon their flow under quasi-one-dimensional environments, they have at least one feature in common: under a particular applied pressure they change their shape to enter and pass through a tube-like (capillary) environment with a diameter smaller than an unperturbed object size\(^{20}\). Establishing the underlying principles of the behavior of soft matter objects with different original, unperturbed dimensions and mechanical properties, which are confined to capillaries with different entrance angles and diameters under various applied pressures can shed light on many of the biological systems described above.

Hydrogel particles with well-defined dimensions, shapes and mechanical properties represent an excellent model system for studies of many soft matter objects in constrained environments. By examining the behavior of micrometer-size hydrogel particles (microgels) passing through microfluidic (MF) channels, important insights have been obtained on the relationship between the microgel mechanical properties\(^{20}\),
shape\textsuperscript{[21]} and interactions with surfaces\textsuperscript{[22–24]} and microgel flow under confinement. The generalized relationship between the properties of soft objects, the geometry of constrained space and the behavior of soft objects in narrow capillaries are yet to be established.

In the current chapter, I described experimental studies of the entrance, passage and the change of volume of agarose microgels entering a narrow capillary with a diameter smaller than the original (unconstrained) microgel dimensions. The goal was to establish universal principles in the behavior of microgels with varying dimensions and mechanical properties translocating through narrow MF channels with varying and entrance angles. In addition, this chapter described the results of studies of the flow of liquid through confined microgels.

### 4.2 Design of microgel flow experiments

A MF channel with a circular cross-section was fabricated in PDMS following the nitrogen gas stream template method described in detail in the Section 2.1 “Preparation of Microfluidic devices in PDMS”. In particular, the diameter, $d$, of the channel-at-large was $d = 110 \pm 5 \ \mu m$, the diameter of the constriction, $d_c$ was $d_c = 43 \pm 2 \ \mu m$, and the entrance angle ($\alpha$) from the channel-at-large to the constriction along $x$-axis was 15°, 45°, or 60°, as shown in Fig. 4.1A. Microgels with agarose concentration 2, 3, 4, or 5 wt% were prepared with diameters in the range from 40 to 120 $\mu m$, using the method described in detail in the Section 2.2.2.1 “Preparation of agarose microgels using a microfluidic droplet generator”. Representative images of microgels in bright field and fluorescence imaging modes are shown in Fig. 4.1B and C, respectively. The experimental setup to study the microgel flow has been described in detail in the Section 2.3 “Microfluidic setup to study flow of a microgel through a PDMS channel”. Briefly, the flow of an individual microgel within the PDMS MF channel was controlled by varying the difference of water level in upstream and downstream reservoirs, and the motion of the microgel was recorded by a high-speed camera and analyzed by a MATLAB program.
Figure 4.1 Schematic of the MF channel and unperturbed agarose microgels. (A) Schematic of the MF channel with a circular cross-section. The diameter, \( d \), of the channel-at-large is 110 ± 5 μm, the diameter, \( d_c \), of the narrow segment (constriction) is 43 ± 2 μm (α of 15°, 45°, or 60°) or 38 ± 2 μm (α = 30°). The length of the constriction is 400 μm. The abscissa \( x = 0 \) denotes the starting point of the constriction. A microgel with an unperturbed diameter of \( D_0 \) moves along the direction indicated by the blue arrow, under the pressure difference applied along the MF device. (B) Representative optical microscopy image of 100 μm-diameter agarose microgels in an aqueous suspension. The scale bar is 100 μm. Inset shows a microgel with an unperturbed diameter of 80 μm in the channel-at-large (\( d = 107 \) μm). The scale bar is 40 μm. (C) Laser scanning confocal microscopy image of an agarose microgel (\( D_0 = 115 \) μm) in the swollen state. The image was taken at the equatorial plane of the agarose microgel labeled with fluorescein isothiocyanate (FITC). The scale bar is 25 μm.

4.3 Studies of liquid flow through a confined microgel

We employed fluorescence recovery after photobleaching (FRAP) in combination with confocal laser scanning microscopy (CLSM) to measure the rate of water flow, \( Q \), through a microgel trapped at the entrance to the constriction, as a function of applied pressure difference \( \Delta P \). Prior to this experiment, CLSM experiments were performed in the microchannel with incremental steps of 5 μm along the \( z \)-direction up to the depth of 200 μm, in order to map a circular microchannel cross-section from the 3D reconstruction of the stacked CLSM images (Figs. 4.2A and B). The design of these experiments is described in detail in the Section 2.1.2.4 “Characterization of the geometry of modified PDMS MF channels”. The FRAP experiments were performed along the \( z \)-direction at the middle position with the highest fluorescent intensity, in order to avoid the attenuation of intensity that occurs in CLSM experiments when \( z \)-coordinate is varied.
Figure 4.2 Determination of the middle plane of a circular microchannel by using a z-stack of CLSM images. (A) Selected fluorescence microscopy images obtained by depth scanning of the microchannel filled with a 0.01 mg/mL of aqueous solution of fluorescein isothiocyanate-dextran by CLSM. A 200 μm-depth scanning was performed at an increment of 5 μm. The respective labels on the images show the position of the imaged plane with respect to the middle plane (labeled as 0 μm). FRAP experiments were conducted for the plane at \( z = 0 \) μm. (B) Circular cross-section of the channel-at-large obtained by 3D reconstruction of 41 images. Scale bar is 200 μm and applies to all images.

Following these experiments, a microgel with an unperturbed diameter \( D_0 = 104 \) μm was introduced in the microchannel \( (d_c = 38 \) μm, \( \alpha = 30^\circ) \) and a pressure difference not exceeding \( \Delta P_{\text{max}} \) was applied to the MF system to confine the microgel in the tapered zone of the microchannel. An intense (100% power of 5.4 mW), 25 sec laser pulse photobleached a rectangular region in the microchannel-at-large using the NIS Element software. To monitor the recovery of fluorescence, the program set the instrument to the attenuated beam (10% power) and a series of 20 images was recorded with 5 sec intervals between image capturing. Photoemission intensity data were collected from the images (Fig. 4.3), and the reference photoemission intensity was obtained from the image taken with the attenuated beam prior to the photobleaching event (see Appendix 2.1 and 2.2 for detailed interpretation).
Figure 4.3 Data analysis of FRAP experimental results. (A) A series of CLSM images obtained at 10% power of laser beam. The labels on the images show respective time after photobleaching event. The top image is captured before the photobleaching event at the same location. $x$ is the flow direction and $r$ is the radial coordinate of the circular cross-section. Scale bar is 100 μm. The calculated value of $M_1/M_0$ with time from (B) is labeled as a vertical white line in each image, with the red line indicating the value of $M_1/M_0$ in the image of $t = 0$ sec. (B) The variation of $M_1/M_0$, the normalized first moment of the fluorophore intensity distribution in the microchannel (see Appendix equation (A2.1) for more details), plotted as a function of time. The solid line is a fit of $M_1/M_0$ vs. $t$ to the linear function $M_1/M_0 = X_0 + Ut$; the average velocity of the liquid in this experiment is $U = 0.35$ μm/s.

4.4 Results and discussion

4.4.1 Microgel translocation through a capillary

In a typical experiment, a microgel with a particular diameter and Young's modulus was introduced in a microchannel comprising a channel-at-large with a diameter $d = 110 \pm 5$ μm, a tapered section, and a constriction with a diameter $d_c$ of $43 \pm 2$ μm or $38 \pm 2$ μm (Fig. 4.1A). The entrance angle, $\alpha$, to the constriction was $15^\circ$, $45^\circ$, or $60^\circ$. We note that in a cardiovascular system, the tapering angle between wide
and narrow blood vessels, that is, between a parent vessel and a daughter branch is typically, lower than 30°, but can reach 70° [25]. The constriction expanded back to the channel-at-large with a diameter $d = 110 \pm 5 \mu m$ via the second tapered section. Both the channel-at-large and the constriction had circular cross-sections [26]. In Fig. 4.1A, the abscissa $x = 0$ denotes the entrance to the constriction. A microgel was forced into the microchannel by applying a pressure difference, $\Delta P$, which was controlled by varying the relative heights of water reservoirs connecting upstream and downstream to the MF device. The motion of the microgel was recorded with a high-speed camera (Canon EX-F1) and analyzed by a code written in MATLAB.

Agarose microgels with an unperturbed diameter, $D_0$, varying in the range from 40 to 120 $\mu m$ and the Young's modulus in the range from 2.6 to 20.2 kPa were prepared by the MF method described elsewhere [27,28]. Fig. 4.1B shows a representative optical microscopy image of 100 $\mu m$-diameter agarose microgels. The particles had a round shape and 4.2% polydispersity (defined as the standard deviation in the dimensions of the microgels divided by the mean diameter). Inset shows an unperturbed 80 $\mu m$-diameter microgel in the channel-at-large.

We verified that polymer concentration is uniform throughout the body of the microgel by covalently labeling agarose molecules with a fluorescent dye fluorescein isothiocyanate and examining the distribution of fluorescence intensity in the microgel using confocal fluorescence microscopy images (Fig. 4.1C). Image analysis was conducted for the microgels in an unperturbed swollen state, where the effect of heterogeneity would be amplified [29]. The relative standard deviation in fluorescence intensity throughout the microgel body was $11.2 \pm 0.9 \%$, indicating that a swollen microgel has a uniform distribution in agarose concentration and structure on the micrometer scale.

When the microgel stopped at a particular pressure difference in the tapered region of the microchannel, the value of $\Delta P$ was incrementally increased in steps of 50 Pa and maintained for 60 sec to stabilize the microgel at the new position along the $x$-axis. Following a repeated increase in $\Delta P$, the microgel moved in a step-wise manner along the tapered zone, until it entered the constriction. At pressure difference $\Delta P$ exceeding $\Delta P_{\text{max}}$ the microgel passed through the constriction. A detailed description of these experiments is given in Chapter 3 and also in our previous work [30].
The shape of the spherical microgel changed under progressive confinement (Fig. 4.4). At small values of \( \Delta P \), the microgel body was localized completely inside the tapered region, with the axial position of its front tip \( x_f < 0 \) (Fig. 4.4A). In this case, the microgel acquired the shape of a truncated-cone with the left and the right caps facing the liquid. As the value of \( \Delta P \) increased, the microgel front entered the constriction \( (x_f > 0) \) (Fig. 4.4B). The constricted part of the microgel acquired a capped cylindrical shape with a diameter \( d_c \). In this situation, as long as the length of the constricted part of microgel was relatively short, the pressure difference across the tapered microgel part exceeded the pressure difference along the constricted microgel portion. At higher \( \Delta P \), the value of \( x_f \) increased, and the pressure difference across the constricted part of the microgel exceeded the difference in pressure along the tapered microgel portion (Fig. 4.4C). The increase in \( x_f \) occurred, until \( \Delta P \) exceeded \( \Delta P_{\text{max}} \), at which the microgel completely entered the constriction and then passed it. In order to localize the microgel completely in the constriction (Fig. 4.4D), the value of \( \Delta P \) was rapidly reduced to zero when the microgel was entirely in the narrow segment of the channel. The changes in the initial shape and volume of the microgel were reversible and completely recovered within 30 sec after releasing it from the constriction\(^{[30]} \), and upon their repetitive insertion in the constriction, it exhibited identical behavior. Furthermore, for each \( \Delta P \), the change in the position and shape of the microgel was reversible and independent on the history of microgel insertion in the constriction. Figs. 4.4A’-D’ show representative optical microscopy images of the microgel in the confined states (corresponding to Fig. 4.4A-D), with a distribution of microgel portions between the tapered region and the constriction.
Figure 4.4 Microgel in different confined states. (A-D) Schematics of the microgel in different positions along the microchannel. (A) Microgel is localized completely in the tapered zone with the axial position of its front tip, $x_f < 0$. (B) A small portion of the microgel is located in the constriction of diameter $d_c$, with a front tip position, $x_f > 0$. The pressure difference across the microgel is dominated by its tapered portion. (C) A large portion of the microgel enters a constriction, with the length $x_f$ of the constricted microgel portion. The pressure difference across the microgel is dominated by its constricted portion. For $x_f > 0$ in (B) and (C), the smallest diameter of the microgel in contact with the microchannel walls is $d_c$. (B) The microgel is localized completely in the constriction. (A'-D') Representative optical microscopy images of the microgel in the confined states corresponding to (A-D). The red line shows the front tip of the microgel. $\alpha = 30^\circ$. The scale bar is 100 μm.

4.4.2 Flow of liquid through a confined microgel

As a microgel travels through the channel-at-large ($d > D_0$), an insignificant water flow occurs through the polymer network, however when it is trapped in the tapered microchannel region, a pressure difference $\Delta P$ across the microgel creates significant water flux through it. For the microgel trapped at the entrance of the constriction, with the different portions of its body in the tapered zone and in the constriction, we performed fluorescence recovery after photobleaching (FRAP) experiments using confocal laser scanning microscopy (CLSM) to measure the rate of water flow, $Q$, as a function of $\Delta P$. Microgels were suspended in 0.01
mg/mL aqueous solution of dextran molecules labeled with fluorescein isothiocyanate (average molecular weight 70,000 g/mol, and diameter[31] 12.0 nm). After introducing a microgel into a microchannel, a pressure difference not exceeding $\Delta P_{\text{max}}$ was applied to the MF system to confine the microgel in the tapered zone of the microchannel. An intense laser pulse was used to photobleach a rectangular 250 $\mu$m $\times$ 150 $\mu$m region in the microchannel-at-large. To monitor the recovery of fluorescence, a series of 20 CLSM images was recorded at the attenuated beam intensity with 5 sec intervals between image capturing. The flow rate of water was calculated from the change in the fluorescence intensity distribution between the images (see Appendix 2.1 and 2.2 for detailed interpretation).

Figure 4.5A shows a non-monotonic variation of the flow rate $Q$ with $\Delta P$: an increase of $Q$ at lower values of $\Delta P$, followed by the reduction in water flow rate $Q$ at higher values of $\Delta P$. Arrows $a'$-$c'$ indicate microgel positions shown in Figs. 4.4A'-C'. We note that at $\Delta P < 2000$ Pa, the contact between the microgel and the channel walls was not conformal and under these conditions, the flow rate of water was dominated by the leakage through the gaps between microchannel walls and the microgel (Fig. 4.6). Significant leakage through the gaps was expected only at $\Delta P < E_0$, where $E_0$ the Young’s modulus of the undeformed gel (see Appendix 2.3 for clarification).

![Figure 4.5](image)

**Figure 4.5 Effect of the pressure difference on the flow rate of water through the confined microgel.** (A) Experimentally measured variation of the volumetric flow rate with pressure difference, plotted for the degree of confinement $D_0/d_c$ of 3.24 ± 0.03. Arrows indicate microgel positions a’-c’ shown in Fig. 4.4. The portion of the microgel confined to the constriction increases with applied pressure difference. $D_0 = 104 \mu$m, $d_c = 38 \mu$m, $E_0 = 2.57$ kPa, $\alpha = 30^\circ$. (B) The same data presented on a log-log plot and compared with two scaling predictions $Q \sim \Delta P$ for weakly-deformed microgel (red line) and $Q \sim \Delta P^{-2.0}$ for constriction-dominated regime (blue line).
Figure 4.6 Effect of pressure difference on the volumetric flow rate of water through the microgel confined at a wider range of position within the MF channel. (A) Variation of the flow rate with the pressure difference, plotted for the confinement $D_0/d_c = 3.24 \pm 0.03$. (B-E) Optical microscopy images of the confined microgel under applied pressure difference of 808, 3258, 6610, and 9721 Pa, respectively. The red line in the images marks the front tip of the microgel. Scale bar is 100 $\mu$m. $D_0 = 104 \mu$m, $d_c = 38 \mu$m, $E_0 = 2.57$ kPa, $\alpha = 30^\circ$.

The non-monotonic variation in the volumetric flow rate of water $Q(\Delta P)$ was explained as follows. In the absence of leakage, the flow rate of water through the microgel is equal to $Q = \Delta P/R$ (similar to Ohm’s law), where $R$ is the hydraulic resistance of the porous microgel. Assuming laminar flow of water through the microgel pores, the hydraulic resistance of a pore of diameter $\xi$ and length $L$ is described by the Poiseuille’s law as $R_\xi \sim \eta L/\xi^4$, where $\eta$ is viscosity of water and the numerical coefficients are dropped. A microgel with length $L$ and diameter $D$ contains $\sim (D/\xi)^2$ such pores connected in parallel with a total hydraulic resistance

$$R \approx R_\xi/(D/\xi)^2 \approx \eta L/(D\xi)^2.$$ (4.1)
At small pressure difference $\Delta P$, the spherical microgel is weakly deformed ($L_0 \approx D_0$) and its resistance to flow $R_0 \sim \eta(D_0\xi_0^2)$ is almost independent of pressure. For this linear regime of weakly-deformed microgels, a linear dependence of the flow rate on the pressure difference is expected, that is, $Q \approx \Delta P / R_0$, as shown with the red line in Fig. 4.5B, with the best fit value of resistance $R_0 \approx 1$ Pa s/μm$^3$ (see Appendix 2.4.6 for detailed explanation).

At a higher pressure difference, the frictional force imposed by the flow of water pulls the microgel from the tapered zone into the constriction. In both regions, the microgel is biaxially compressed in the radial directions and elongated along the axial direction, however the shape of the microchannel makes this elongation non-uniform: the back end of the microgel in the tapered region is less elongated than its front end in the constriction. The position $x_f$ of the front end of the microgel in the constriction is calculated by balancing the frictional force (imposed by the water flow on polymer chains) by osmotic force due to excluded volume repulsion and the elastic force due to the connectivity of the deformed polymer network, resulting in the following numerical result, which matches well with experiments.

$$x_f \sim \Delta P^{2.3}$$

(see the red lines in Fig. 5B). The osmotic pressure $\pi$ originating from the excluded volume repulsion between the polymer chains of the strongly confined microgel is proportional to thermal energy $kT$ per pore volume $\xi^3$, where $\xi$ is the correlation length in the constriction. The osmotic pressure $\pi$ is determined by the applied pressure difference $\Delta P \approx \pi \approx kT/\xi$, and the pore size of the microgel decreases with increasing pressure as

$$\xi \approx (kT/\Delta P)^{1/3}. \quad (4.3)$$

The strongly compressed portion of the microgel in the constriction dominates the flow resistance $R$ that can be estimated from equation (4.1) as

$$R \approx \eta x_f / (d_c \xi)^2 \sim \Delta P^{3.0}. \quad (4.4)$$

Here, the length of the flow resistance-dominating region is taken as the length of the constricted portion of the microgel $L \approx x_f$ (equation (4.2)), the width of the flow resistance-dominating region is the diameter of the constriction $D \approx d_c$, and the correlation length in constriction is approximated by equation (4.3).
strong increase of flow resistance with the applied pressure difference $\Delta P$ in the constriction-dominated regime (equation (4.4)) results in the decrease of flow rate

$$Q \approx \Delta P / R \sim \Delta P^{-2.0}.$$  

(4.5)

A sharp decrease of flow rate with increasing pressure difference (a blue line in Fig. 4.5B with the slope of $-2.0$) is in agreement with experimental observations. Therefore, the crossover from the tapering regime of weakly-deformed microgel with approximately constant flow resistance $R_0$ and linear $Q-\Delta P$ relationship to the constriction regime of the strongly-confined microgel with flow resistance rapidly increasing with pressure difference (equation (4.4)) explains the non-monotonic pressure dependence of flow rate shown in Fig. 4.5.

The non-monotonic effect of pressure on the flow rate of liquid through the geometrically constrained microgel is one of the most interesting and important findings of this work. The flux of water was determined by the location of microgel in the capillary and as such, was controlled by the applied pressure difference. At the moderate pressure drop, the flux of water was dominated by the almost constant hydraulic resistance of the microgel portion in the tapered confinement, and the flow rate of water increased with increasing pressure difference. As the microgel progressed further into the constriction region, the hydraulic resistance was rapidly increasing dominated by the constricted microgel part, and water flux reduced with increasing applied pressure difference.

### 4.4.3 Microgel progression into confinement

With an increasing pressure difference $\Delta P$, the microgel moves from the tapered zone into the constriction, as illustrated in Fig. 4.4. Figure 4.7A shows the change in the position of the front microgel tip, $x_f$, plotted as a function of the applied pressure difference, $\Delta P$, under the same degree of microgel confinement (defined as the ratio $D_0/d_c$). The negative values of $x_f$ (Fig. 4.7A, left-most three blue symbols) correspond to the microgel located completely in the tapered region.

For the same degree of confinement, softer microgels with a lower Young’s modulus required a smaller pressure difference $\Delta P$ to move along the $x$-direction to enter the constriction. In Fig. 4.7B, the data from
Fig. 4.7A are re-plotted in the normalized form: the position of the front microgel tip $x_f$ was normalized by the constriction diameter $d_c$, while the pressure difference $\Delta P$ was normalized by the Young's modulus $E_0$ of an unperturbed microgel of diameter $D_0$. Under the same degree of confinement $D_0/d_c$, the three sets of data points obtained for the microgels with different moduli $E_0$ overlapped to form a master curve for the variation in the relative microgel position $x_f/d_c$ vs. the normalized pressure difference $\Delta P/E_0$. The master curve illustrates that during microgel progression through the confinement, its position under a particular pressure difference was controlled by the rigidity and the size of the undeformed microgel.

The black curve in Fig. 4.7B shows the results of the numerical force balance calculation for the normalized position $x_f/d_c$ (a single fitting parameter $\kappa = 0.365$ was used to account for the neglected effects of out of plane bending of $y$-$z$ slices of the microgel and the deformation of the microgel caps – see Appendix 2.4 for detailed interpretation). Figure 4.7B shows an excellent agreement between the numerical results and the experimental data.

Microgel progression from the tapered region to the constriction was rationalized as follows. When a large portion of the microgel was strongly constricted (Fig. 4.4C), the frictional forces pulled the constricted front portion of the microgel deeper into the constriction, while the wall force acting on the tapered back part balanced it. The force balance between the two portions of the microgel localized in the tapered and constriction regions led to equation (4.6) (a normalized form of equation (4.2))

$$x_f/d_c \sim (\Delta P/E)^{2.3} \quad \text{for} \quad x_f > 0$$

in the constricted regime (see Appendix Fig. A2.4E for the origin of 2.3). This scaling form, plotted as a red line in the inset of Fig. 4.7B, is in agreement with the experimental data for the significantly constricted microgel.
Figure 4.7 Dependence of the position of the microgel on the applied pressure difference. (A) Effect of pressure difference on the position of the microgel front tip $x_f$ along the $x$-axis in the microchannel, plotted at the same degree of confinement $D_0/d_c = 2.24 \pm 0.01$ for the microgels with the Young’s modulus of 2.57 (●), 8.26 (■) and 12.54 kPa (◆) (corresponding to agarose concentration in the microgel of 2, 3, and 4 wt%, respectively). The results are obtained for microgels with a diameter $D_0$ of 94, 101, and 100 μm, which were passing through the constriction with a diameter $d_c$ of 42, 45 and 45 μm, respectively, at $\alpha = 15^\circ$. (B) A master curve of the relative microgel position $x_f/d_c$, plotted as a function of $\Delta P/E_0$. The colors of symbols in (B) are the same as in (A). The black curve shows the result of numerical calculations with a fitting parameter $\kappa = 0.365$ (see Appendix Fig. A2.4E). Inset shows the data for positive $x_f$ on double-logarithmic scale. Red line with a slope 2.3 demonstrates agreement of data with scaling equations (4.2) and (4.6) for the constriction regime ($x_f > 0$).

4.4.4 Microgel passage through the constriction

At high pressure difference $\Delta P > \Delta P_{\text{max}}$, the wall force acting on the tapered part of the microgel can no longer balance the friction force acting on the constricted part, and the microgel passes through the constriction (later in the text $\Delta P_{\text{max}}$ is referred to as a translocation pressure).

This effect was examined for the varying degrees of confinement of the microgels with different mechanical properties in microchannels with different entrance angles. Fig. 4.8A shows the variation in $\Delta P_{\text{max}}$ vs. $D_0$ for the microgels with different Young's moduli $E_0$ in the initial swollen and unperturbed state. For a particular value of $E_0$, with increasing dimensions of the microgel, a greater pressure difference was required to force it into the constriction, owing to the stronger shape and volume change experienced by larger microgels. For a particular microgel diameter, the translocation pressure $\Delta P_{\text{max}}$ was greater for microgels with a higher value of Young's modulus, since it was harder to deform a microgel with a higher stiffness. Figure 4.8B shows the dependence of the ratio $\Delta P_{\text{max}}/E_0$ on the degree of microgel confinement.
For each geometry of microchannel studied, that is, the entrance angle \( \alpha \), all the data points collapsed onto a master curve, which combined four experimental parameters \( D_0, d_c, E_0 \), and \( \Delta P_{\text{max}} \). This result implies that for any set of three parameters \( D_0, d_c, E_0 \), or \( \Delta P_{\text{max}} \), the other characteristics can be obtained from the master curve. Importantly, the master curve could be used to measure the Young's modulus of gel particles with different rigidity by examining their translocation pressure \( \Delta P_{\text{max}} \) as a function of the degree of confinement.

Theoretically, the translocation pressure \( \Delta P_{\text{max}} \) scales with the degree of confinement as

\[
\frac{\Delta P_{\text{max}}}{E_0} \sim (D_0/d_c)^{14/3}
\]  

(4.7)

(the derivation of equation (4.7) and \( \alpha \)-dependent coefficient of proportionality is given in Appendix 2.4.5.2). The solid lines in Fig. 4.8B show the scaling form of equation (4.7) and match the experimental results.

Larger values of \( \Delta P_{\text{max}}/E_0 \) were required to bring the microgel into the constriction with a larger tapering angle \( \alpha \) (Fig. 4.8B). This result agreed with an earlier work performed for the limited range of entrance angles (3° and 6°), in which the difficulty in the transit of cells with an increasing entrance angle was established experimentally and theoretically\cite{33,34}. With an increasing entrance angle \( \alpha \), the x-component of the wall force exerted on the tapered portion of the microgel increases proportional to \( \tan \alpha \), at small \( \tan \alpha \ll 1 \), which requires a stronger friction force in the constricted part at the translocation point. Hence the pressure difference \( \Delta P_{\text{max}} \) required to force the microgel into the constriction increases with the entrance angle \( \alpha \) to the constriction.
Figure 4.8 Dependence of $\Delta P_{\text{max}}$ on the degree of microgel confinement. (A) Variation in $\Delta P_{\text{max}}$ plotted as a function of microgel diameter $D_0$. $d_c = 43 \pm 2 \, \mu m$. Microgels with Young’s modulus of 2.57 kPa (circle symbols), 8.26 kPa (square symbols), 12.54 kPa (diamond symbols) and 20.21 kPa (triangle symbols) (corresponding to agarose concentration in the microgel of 2, 3, 4 and 5 wt%, respectively) were tested in the constriction with the entrance angle $\alpha = 15^\circ$. (B) Log-log plot of the master curves describing the relationship between $\Delta P_{\text{max}}/E_0$ and the degree of microgel confinement $D_0/d_c$ in microchannels with different entrance angles $\alpha$. Solid lines are the theoretical results obtained from equation (4.7) with a slope 14/3. Microgels with Young’s moduli (denoted as in (A)) were tested in constrictions with the entrance angles $\alpha$ of 15° (blue color), 45° (red color), and 60° (green color).

4.4.5 Change in microgel volume in the confined state

Upon complete microgel entrance into the constriction, its equilibrium volume, $V$, at $\Delta P = 0$, reduced in comparison with its original unperturbed volume, $V_0$, implying that water was partly expelled from the microgel interior. We examined the change in microgel volume under confinement, $V/V_0$, for the microgels with different Young’s moduli as a function of the degree of their confinement, $D_0/d_c$. Figure 4.9A shows that with an increasing degree of confinement, the ratio $V/V_0$ decreased, due to a stronger compression of larger microgels and expulsion of a larger amount of water from their interior. Importantly, for the same values of $D_0/d_c$, the relative change in microgel volume was not affected by a ~ 10-fold change in Young’s modulus, implying that microgels with different deformabilities have the same Poisson’s ratio and the reduction in $V/V_0$ exhibits the same dependence on the degree of confinement $D_0/d_c$. Since microgel rigidity was tuned by changing polymer content, we conclude that the relative reduction in microgel volume in confinement does not depend on polymer concentration and cross-linking density.

Importantly, a similar variation $V/V_0$ vs. $D_0/d_c$ was observed for all microgels confined to a constriction with varying entrance angles (Fig. 4.9B). This similarity indicates that the values of $V$ and $V_0$ were measured in equilibrium states and the relative change of the microgel volume was independent on the history of microgel progression in the constriction.

We developed a quantitative model for the change in microgel volume in confinement, by considering a uniform equi-biaxial compression of a microgel, upon its complete entrance into the constriction. The increase in osmotic pressure in the microgel (due to the increase in polymer concentration) is partially relaxed by microgel elongation in the unconstrained direction. This elongation, in turn, creates an elastic
stress in the axial direction. At an equilibrium state, the elastic stress built up in the polymer network is balanced by the osmotic pressure, which results in the relative volume decrease

\[
\frac{V}{V_0} = \frac{(5/7)^{1/3}(D_0/d_c)^{-4/3}[(1/2 + v)^{1/3} + (1/2 - v)^{1/3}]},
\]

(4.8)

where \( v = [1/4 - (8/4725)(D_0/d_c)^{-10}]^{1/2} \) (see Appendix 2.4.5.1). These relations are represented by the black curves in Fig. 4.9A and B. The experimental and theoretical results were in excellent agreement without any fitting parameters. Comparison of the reduction in volume of the cylindrical and spherical microgels showed a \( \sim 10\% \) difference in the variation of \( V/V_0 \) with \( D_0/d_c \).

![Figure 4.9 Effect of the degree of confinement on the change in microgel volume.](image)

(A) Ratio of the final-to-initial volume, \( V/V_0 \) of the confined microgel, plotted as a function of the degree of microgel confinement, \( D_0/d_c \). \( V \) is the volume of the microgel completely localized in the constriction and maintained there for 5 min. The Young’s moduli of the microgels are 2.57 (●), 8.26 (■), 12.54 kPa (◆), and 20.21 (▲) (corresponding to agarose concentration in the microgel of 2, 3, 4 and 5 wt%, respectively). The black line gives the theoretical result, equation (4.8), for \( V/V_0 \). (B) Variation in volume ratio for microgels confined to the constrictions with different entrance angles.

### 4.4.6 Discussions

For the different degrees of confinement of microgels with varying mechanical properties in capillaries with different entrance angles we established the underlying principles for (i) the flux of liquid through the confined microgel; (ii) the microgel's location in the constriction under a particular applied pressure difference; (iii) the critical applied pressure difference beyond which the microgel passes the constriction and (iv) the loss of water by the confined microgel. The position, the applied translocation pressure
difference, and the change in volume of the confined gel, when normalized appropriately, depend only on the degree confinement and the geometry of tapered region, and are independent of the polymer concentration in the gel in its undeformed state. The experimental results were in agreement with the theory developed for non-linear biaxial deformation of unentangled polymer gels. We also show a non-monotonic change in the flow rate of liquid through the constrained microgel, governed by its progressive confinement.

Our model shows that these results are universal for unentangled gels formed by flexible polymer chains, swollen in a good solvent and cross-linked permanently on the time scale of experiments. As long as these criteria are met, the behavior of the gel does not depend on the chemical composition of the polymer or the solvent.

The results of our work are relevant and important for the development of embolization therapies which utilize deliberate obstruction of blood vessels with gel beads and are used for treatment of hemorrhage or specific types of cancer\cite{13,35,36}. In our work, the degree of confinement of microgels was from 1.1 to 2.4, that is, it was comparable with the range of $1.4 \pm 0.38$ to $3.1 \pm 1.04$ encountered in embolization therapies for occlusion of blood vessels\cite{13,16,35–37}. On the other hand, there is no limitation in our model that precludes the use of larger degrees of confinement, both experimentally and theoretically, which underlines the universality of our model system.

Furthermore, our work offers a truly unique capability to mimic occlusion of blood capillaries in embolic strokes which account for $\sim 25\%$ of all strokes\cite{38}. In embolic strokes, thrombi comprising fibrin gel travel from distant locations in the body to the brain, where they obstruct flow of blood in a narrow blood vessel. In the occluded vessel, the clot experiences deformation, loss of water and change in structure. These are the features reproduced in our model. According to the recent scientific evidence, change in fibrin gel structure\cite{39}, gel biomechanical properties\cite{40}, and strain\cite{41} strongly influence the susceptibility of occlusive thrombi to thrombolysis. The microfluidic approach described in the present work offers the ability to study these effects. In our work, the microgels were constrained in the capillaries under applied pressure difference from their swollen, unperturbed state, in contrast with previous studies, in which gels were formed directly in the narrow capillary by gelling a precursor solution\cite{42–44}. For biological systems with a higher complexity, e.g., for thromboembolism or the flow of blood cells under confinement, our results may help in gaining understanding of the physical principles governing the system behavior. The predictive
power of our results for such systems is yet to be tested. Finally, the non-monotonic change in flow rate of liquid through the occlusive microgel has far-reaching implications for the diffusion-driven vs. convection mediated mechanism of thrombolysis achieved by supplying a solution of a thrombolytic drug to the clot occluding a blood vessel[45].

The theoretical approach developed in the present work can be extended to polymer gels that belong to other universality classes, including gels that are formed by (i) semi-flexible or rigid polymer chains, (ii) entangled chains, (iii) temporary cross-linkers, and (iv) gels swollen with theta-solvents or – in case of semi-flexible chains – marginal solvents. Based on the established dependence of elastic and osmotic energies on equilibrium polymer concentration, these gels will exhibit analogous trends under confinement, but with different scaling powers for the collapse of normalized data into master curves. For example, the change in volume upon confinement of unentangled gels formed by flexible chains in a theta solvent[46] is expected to follow $V/V_0 \approx (D_0/d_c)^{-1}$, that is, with exponent -1, instead of -4/3, characteristic for gels swollen by a good solvent (see equation (4.8)).

We note that in confined gels, stresses are distributed over the entire gel volume. In contrast, for droplets or objects composed of a liquid core encapsulated by a membrane, e.g., vesicles and polymer capsules, deformation-induced stresses are manifested only at the interface[47–49]. An interface between a droplet and a continuous phase is described by interfacial tension, and for a particular degree of confinement, the translocation pressure difference will depend only on the geometry of the tapered region[47]. For vesicles and polymer capsules, interfacial stresses depend on multiple interfacial properties, e.g., bending, shear and area dilatation moduli, all of which would have to be accounted for, in order to collapse the normalized data into master curve[49]. Another important exception is the biological cell that can exhibit both active and passive (pressure-driven) mechanisms of passage through narrow capillaries, with its volume conserved during deformation[50,51].

Importantly, the generalized relationship between the translocation pressure, the degree of microgel confinement and the entrance angle to the capillary enables the determination of the Young’s modulus of hydrogel particles, complementary to other techniques[52,53], or the translocation pressure of hydrogel objects with known elastic properties. The latter feature result has implications in the biomedical field.
4.5 Conclusions

In this chapter, we reported an experimental and theoretical study of translocation of micrometer-size hydrogels (microgels) through microfluidic channels with a diameter smaller than an unperturbed microgel size. For microgels with different dimensions and mechanical properties, under a range of applied pressures, we established the universal principles of microgel entrance and passage through microchannels with different geometries, as well as the reduction in microgel volume in confinement. The principles have implications for a broad range of phenomena, including occlusion of blood vessels by thrombi and needle-assisted hydrogel injection in tissue engineering.

The unexpected variation in liquid flow through a confined has implications for convection-enhanced permeability of fibrinolytic agents through occlusive blood clots (thrombi), which may greatly improve the efficiency of therapeutic treatment of patients with diseases such as ischemic strokes or acute pulmonary embolism. The MF platform described in this chapter, as well as Chapter 3 was further implemented to studies of embolism and of artificial occlusive blood clots, as described in Chapter 5.
References


Chapter 5
A microfluidic model for \textit{in vitro} studies of thromboembolism and thrombolysis

5 A microfluidic model for \textit{in vitro} studies of thromboembolism and thrombolysis

5.1 Introduction

Obstruction of blood vessels with pathological blood clots (thrombi) is a primary reason for ischemic strokes, pulmonary embolism and myocardial infarction, the diseases that are the leading causes of long-term disability and death worldwide\cite{1-4}. Due to the prevalence and severe clinical consequences of these diseases, research efforts have focused on developing efficient strategies that rapidly restore blood flow through an occluded vessel\cite{5,6}.

Two main approaches used for recanalization of blood vessels include intravenous administration of thrombolytic drugs\cite{7,8} or endovascular therapy utilizing e.g., a guidewire or a catheter to remove an obstructive clot. Importantly, the latter method can be also assisted by the administration of thrombolytic drugs\cite{6,9-11}. Thus the proteolytic clot dissolution is vitally important in both types of treatment of thromboembolism.

Thrombi contain 30 - 80 \% of a three-dimensional filamentous fibrin gel\cite{12,13} formed by the lateral and longitudinal association of protofibrils into branching fibers that organize into a three-dimensional network\cite{14}. A plasma transglutaminase, Factor XIIIa, covalently crosslinks fibrin to form a mechanically and chemically stable fibrin clot. Thrombolysis leads to the dissolution of fibrin gels in a series of enzymatic reactions occurring due to the colocalization of the plasminogen activator with plasminogen on the surface of fibrin, subsequent activation of plasminogen, and the resulting digestion of fibrin\cite{7,8,14}. New knowledge generated over the past decade suggests that occlusive fibrin clots are particularly resistant to lysis, presumably, due to the hampered activation of plasminogen and strain-induced changes in fibrin gel structure\cite{15-17}. For example, suppressed plasminogen activation and plasmin generation were observed in strained clots, and the lysis front was blocked within an hour\cite{15}. The rate of dissolution of fibrin clots reduced ten-fold when the strain was 177\%\cite{17}.
Strain-induced changes in fibrin gels have been comprehensively studied by using in vitro models for macroscopic systems\(^{18,19}\). It has been established that under extension or axial shear, changes in gel structure occurred on multiple length scales, including fibrin molecules, individual fibers, fiber network, and the entire fibrin clot. On the molecular scale, gel stretching resulted in \(\alpha\)-helix to \(\beta\)-sheet transition of the secondary structure of fibrin, unfolding of the coiled coil region in fibrin monomers and exposure of their hydrophobic residues to water\(^{18}\)\(^{20}\). On the nanometer scale, axial shear stress rendered the alignment of fibrin fibers and densification of their network\(^{15}\). On the macroscopic level, stretched fibrin gels dehydrated and significantly reduced in volume\(^{18}\).

These studies have been conducted in assumption that upon confinement in narrow blood vessels occlusive clots undergo strong elongation determining changes in clot structure. Compression of fibrin gels was largely unexplored. Recent studies of uniaxial compression of macroscopic fibrin gels revealed a nonlinear mechanical response\(^{21}\) and spatial non-uniformity of fibrin network\(^{22}\) originating from fiber stretching, bending, and buckling, as well as fibrin network densification. Furthermore, experiments conducted for unconfined clots grown in tubes\(^{15,17,18}\) or perfusion cells\(^{23}\) mimic only some of in vivo systems, however they fail to reproduce others, e.g., embolic strokes, in which a blood clot formed in a distant location of the body travels through the arteries, until it is lodged in a blood vessel with a diameter significantly smaller that the clot size\(^{24,25}\). Upon confinement, the clot undergoes a three-dimensional deformation, that is, a bi-axial compression imposed by the vessel walls and unidirectional elongation in the unconstrained direction. Studies of confinement-induced changes in structure, dehydration and lysis of occlusive fibrin gels can shed light on changes in structure and properties of obstructive thrombi and their implications for the rate of fibrinolysis.

With regards to the therapeutic thrombolysis, a drug introduced into the vasculature is delivered to the thrombus by flowing blood, with its perfusion and diffusion into the occlusive clot. Current in vitro studies generally rely on the sole diffusion mechanism for the drug introduced at the edge of a pre-formed clot\(^{26,27}\). The development of a flow-governed model for in vitro studies of lysis would account for the flow-induced delivery and permeation of clots with a drug. Such a model would enable the evaluation of lysis efficiency by observing the changes in clot structure and measuring enhanced flow of the liquid through the clot. Microfluidics (MFs) shows promising applications in studies of clot rupture and the delivery of its
fragments to block another vessel\textsuperscript{[28,29]} or for the screening of an anti-thrombotic drug activity\textsuperscript{[30,31]}, however the applications of MFs in studies of thromboembolism and thrombolysis have not been explored.

Here, we report a new MF platform for \textit{in vitro} studies of thromboembolism and thrombolysis. The model utilizes (i) fibrin hydrogel particles (microgels) with controlled dimensions, compositions, structures and mechanical properties; (ii) flow-induced insertion of a microgel into a narrow segment of the microchannel and (iii) flow-induced delivery of thrombolytic drug to the obstructive fibrin microgel. In comparison with existing studies of thromboembolism and thrombolysis, the proposed model offers two new, critically important features: it mimics biaxial compression and unidirectional elongation experienced by blood clots \textit{in vivo} and the capability to study obstructive behavior and lysis of clots under flow conditions.

5.2 Preparation of fibrin microgels

5.2.1 Materials

All chemicals, unless specified, were purchased from Sigma-Aldrich Canada. Fluorinated oil HFE-7500 3M Novec was purchased from 3M. The triblock copolymer surfactant of perfluoropolyethers and a poly(ethylene oxide) polypropylene copolymer [PFPE-P(EO-PO)-PFPE] was synthesized as described previously\textsuperscript{[32]}. Fluorescein (FITC)-human fibrinogen was prepared by modifying human fibrinogen (Enzyme Research Laboratories) by (5-(and-6)-carboxyfluorescein succinimidyl ester (5(6)-FAM NHS, Invitrogen). 143 µL 10mg/mL 5(6)-FAM NHS in dry DMSO was added to the 5 mL 25 mg/mL fibrinogen solution (0.1 M NaHCO\textsubscript{3} buffer, pH = 8.0) under magnetic stirring under room temperature. After the mixture was stirred for 1 h in the dark at room temperature, 100 µL of 1 M ethanolamine in 0.1 M NaHCO\textsubscript{3} buffer was added into reacting mixture for 15 min to stop the reaction. Finally, the FITC-fibrinogen solution was dialyzed using Tris buffer saline (TBS, 20 mM Tris, 150 mM NaCl, pH 7.4) at 4 °C in the dark, and the TBS was replaced every 24 h until there is no fluorescein in the TBS. Active site-blocked thrombin was prepared by incubating thrombin (Enzyme Research Laboratories) with a 50-fold molar excess of Phe-Pho-Arg-chloromethyl ketone (PPACK; Molecular Innovations), followed by 4 dialysis exchanges at 4 °C into
TBS to remove excess PPACK. AlexaFluor633-labeled t-PA was provided by Prof. Weitz’s lab in McMaster University. All chemicals were used as received without purification.

5.2.2 Microfluidic preparation of microgels

The fibrin microgels were prepared by flow-focusing microfluidic devices\(^{[33]}\) (Fig. 5.1A). Bovine fibrinogen (60 mg/mL in TBS, containing 50 mM Tris, 150 mM NaCl, pH 7.6) solution, Tris buffer and bovine thrombin solution (5U/mL in 10 mM CaCl\(_2\) solution) were injected in the microfluidic devices using two independently controlled syringe pumps (PhD 200 Harvard Apparatus PHD 2000 Syringe Pump, USA) as constituents of the droplet phase. The continuous phase, fluorinated oil containing 1% surfactant PFPE-P(EO-PO)-PFPE was injected into devices by using another syringe pump. The droplets of fibrin precursor were generated on the orifice of microfluidic device, and travelled towards the outlet of device, finally were collected in a 15 mL centrifuge tube containing fluorinate oil by using an outlet tubing. The droplet loading centrifuge tube was put in the 37 °C water bath for 1 h to complete the gelation of fibrin microgels. Enzymatic catalytically mediated polymerization of fibrinogen mixed with thrombin in the droplets resulted in the formation of fibrin gel particles. Later in the text, we refer to these particles as to "microgels".

FITC-fibrin microgels (Fig. 5.1B) were prepared using the same experimental setup. The mixture of bovine fibrinogen solution and FITC-human fibrinogen (60 mg/mL in Tris buffer), instead of bovine fibrinogen was supplied to the microfluidic devices.

In order to transfer the fibrin microgels into the TBS, the emulsion of fibrin microgels was consecutively washed by centrifuge at speed of 1000 rpm for 2 min with fluorinated oil containing 20 % per-fluoroctanol, hexane containing 0.5 % span 80, hexane, 0.1% Triton X water solution, and TBS, finally the fibrin microgels were dispersed in Tris buffer containing 0.02% Tween 20. An aqueous dispersion of the microgels was stored in the fridge for approximately, 1 - 2 weeks before the experiments.
Figure 5.1 Microfluidic generation of fibrin microgels. (A) Schematic of the MF preparation of fibrin microgels. (B) Fluorescence optical microscopy image of FITC-labeled fibrin microgels suspended in the TBS. Scale bar is 500 μm. Inset: individual FITC-labeled fibrin microgel. Scale bar is 100 μm. (C) Size distribution of fibrin microgels dispersed in TBS. In (B, C) $Q_c = 1.5$ mL/h and $Q_{tot} = 0.3$ mL/h, respectively. The flow rates of each of the individual aqueous streams $Q_{fibrinogen}$, $Q_{thrombin}$, and $Q_{buffer}$ is 0.1 mL/h. The distribution is based on the analysis of 100 microgels. (D) Variation in the diameter of fibrin microgels dispersed in TBS, plotted as a function of the flow rate of the F-oil phase, $Q_c$ at $Q_{tot} = 0.3$ mL/h. For each experimental point 50 microgels have been analyzed.

5.3 Characterization of microgel properties

5.3.1 Quantification of fibrin microgel compositions

The compositions of fibrin microgels were quantified in assumption that droplets and microgels have a similar composition and was achieved by analyzing the pixel intensity of precursor droplets loaded with Methylene Blue. A calibration curve was generated by relating the pixel intensity values of droplets
generated by MF emulsification to the concentrations of Methylene Blue in the solution (Fig. 5.2A). In order to determine the concentration of fibrinogen, the droplets containing a mixture of the fibrinogen solution with 1 mM methylene blue, PPACK blocked thrombin, and TBS were generated by varying the ratio of flow rates of the fibrinogen and thrombin solutions, while keeping the flow rate of TBS constant. The images of the precursor droplets were captured using a QIMAGING-QIClick camera. The pixel intensity in the droplets was determined using Matlab and corrected by subtracting the pixel intensity value of the background. For the determination of thrombin and calcium concentrations, the same method was used except for loading 1 mM Methylene Blue in the thrombin solution.

The effect of solution viscosity on experimentally determined fibrinogen and thrombin concentration in the microgels was close to the theoretical prediction based on the assumption of mass conservation and no shrinkage losses resulting from liquid mixing (shown as symbols and solid lines, respectively, in Fig. 5.2B), although a higher viscosity of the fibrinogen solution contributed to a slightly higher concentration of fibrinogen and lower concentration of thrombin in the droplets.

Figure 5.2 Characterization of the concentration of fibrinogen and thrombin in precursor droplets. (A) Calibration curve (red line) obtained by linear regression fitting the light intensity (black symbols) of droplets with varying concentration of Methylene Blue. (B) Variation in the concentration of fibrinogen (red symbols) and thrombin (blue symbols) in the precursor droplets, plotted as a function of the ratio of $Q_{\text{fibrinogen}}/Q_{\text{total}}$. $Q_c = 0.8$ mL/h, $Q_{\text{buffer}} = 0.1$ mL/h, and $Q_{\text{total}} = Q_{\text{buffer}} + Q_{\text{fibrinogen}} + Q_{\text{thrombin}} = 0.3$ mL/h. Lines with respective color show the theoretical prediction of concentration of fibrinogen and thrombin in the precursor droplets, based on the assumption of mass conservation and no shrinkage losses resulting from liquid mixing.
5.3.2 Characterization of nanostructures of fibrin microgels

The nanostructure of the fibrin microgels was examined using scanning electron microscopy (SEM). The fibrin microgels were fixed on the silicon wafers by 5 wt% glutaraldehyde in TBS for 2 h. This silicon wafer with fibrin microgels was placed in a microporous specimen capsule (30 μm pore size, Canemco-Marivac), and water in the microgels was gradually replaced with ethanol by consecutively submerging the capsules in 20, 40, 60, 80, and 100% (v/v) ethanol/water mixtures. The capsule was placed in an Autosamdri-810 Tousimis critical point drier, in which the ethanol was exchanged with liquid CO₂. The liquid CO₂ was brought to a supercritical state. Slow venting of the chambers produced the dried samples, which were gold sputtered using a SC7640 High Resolution Sputter Coater (Quorum Technologies) for 15 sec at 2.0 kV and 20 mA. Subsequently, the gold-coated samples were imaged by Scanning Electron Microscopy (SEM) using a Quanta FEI Scanning Electron Microscope.

5.4 Fabrication of PDMS microfluidic devices

5.4.1 Materials

Hexamethyldisiloxane (HMDS, ≥ 98%), trichloro(1H,1H,2H,2H-perfluoroctyl)silane (97%), and fluorescein isothiocyanate-dextran (FITC-Dextran) (MW = 70 kDa) were purchased from Sigma-Aldrich Canada (Oakville, ON). Silicon wafers were purchased from Wafer World, Inc. (West Palm Beach, FL). SU-8 2025 photoresist and SU-8 developer were from MicroChem Corp. (Newton, MA). AZ40XT photoresist and MIF-300 developer were from Integrated Micro Materials (Argyle, TX). Dow Corning Sylgard 184 Silicone elastomer kit consisting of poly(dimethylsiloxane) (PDMS) prepolymer and crosslinker was from Ellsworth Adhesives Canada (Burlington, ON). All chemicals were used as received without purification.
5.4.2 Fabrication scheme

Before fabrication, two photomasks were designed to produce a three-level MF channel with various diameters in respective level. The first photomask consists only the 1st level of the channel (Fig. 5.3A), with a width of 300 μm and a length of 3.3 mm. The second photomask consists of 1st level with the same dimension, and also the second (width of 200 μm; length of 1 mm) and the third level (width of 50 μm; length of 1.5 mm) (Fig. 5.3B). A shallow angle (15º) connecting different levels was chosen in order to achieve a smooth structural transition during reflow.

Figure 5.3 shows the schematic of the fabrication of the MF devices using soft lithography combined with a reflow phenomenon of a double-layered positive photoresist. The surface of a Si wafer was treated with HMDS at 75 °C for 40 min to render the surface hydrophobic. To prepare the first layer of the patterned photoresist, a positive photoresist was spin-coated on the Si wafer at 500 rpm for 30 sec, and the solvent was evaporated for 1 h. A three-stage baking step was then followed at 70 °C for 5 min, 90 °C for 5 min, and 115 °C for 5 min. The positive photoresist on the Si wafer was exposed to UV-light for 70 sec with the photomask shown in Fig. 5.3A, post-baked at 100 °C for 30 sec, and developed with MIF-300 developer using puddle development process with a total development time of 5 min. During the puddle mode, MIF-300 developer was poured onto the stationary wafer with photoresist that was allowed to sit motionless for 1 min, and the wafer was then spin rinsed and dried. A total of 5 cycles of the puddle procedure above was applied to achieve a complete development of the positive photoresist on the silicon wafer. To increase the height of the photoresist pattern, the silicon wafer with photoresist structure was first, heated at 75 °C for 15 min to remove access water left from the development. Subsequently, positive photoresist was spin-coated at 1,000 rpm for 30 sec on the developed photoresist and baked using the procedure described above. The photomask shown in Fig. 5.3B was then aligned with the previously prepared patterns under the mask aligner, followed by the UV exposure of 70 sec, post-baking at 100 °C for 30 sec, and the puddle development process with a total development time of 5 min. The semi-cylindrical positive photoresist was then reshaped from its rectangular cross-section via a three-stage baking step, i.e., 70 °C for 5 min, 90 °C for 3 min, and 130 °C for 70 sec (Fig. 5.3C). After silanization treatment of photoresist with trichloro(1H,1H,2H,2H-perfluorooctyl)silane at 75 °C for 1 h, a PDMS prepolymer and PDMS curing agent, mixed at a volume ratio of 5:1, were poured onto the photoresist-covered Si wafer, and cured in an oven at
73 °C for 3 h. The cured PDMS was then peeled off from the Si wafer, with the cross-section of the PDMS microchannel being a concave semi-circle. The bonding of two such PDMS sheets was achieved by treating their surface for 10 sec with a hand-held corona treater[34] and alignment of the two PDMS layers under the microscope to form a channel with a circular cross-section (Fig. 5.3C). The sandwich of the PDMS two layers was left undisturbed in an oven at 115 °C overnight to achieve strong bonding.

The geometry of the cross-section of the PDMS replica from the semi-circularly shaped photoresist and that of the aligned and bonded PDMS MF device is shown in Fig. 5.4. A typical MF channel used to study occlusive and relaxation microgel behavior, as well as liquid flow through confined microgels, comprises a 1st level region (“channel-at-large”), in which the spherical shape of the microgel remains unperturbed, a circular 2nd level region \( d = 193 \, \mu \text{m} \) through which the microgel quickly travels, and a constriction with a diameter \( d_c = 65 \, \mu \text{m} \). The constriction symmetrically expands back to the 2nd level region and then to the 1st level region at the exit of the MF channel. Both the 2nd level region and the constriction have circular cross-sections, and the 1st level region has an elliptical cross-section (width of 315 \( \mu \text{m} \); height of 239 \( \mu \text{m} \)) with an aspect ratio of 1.32. A smooth transition between the channel-at-large and the constriction is shown in a fluorescent microscopic image of the MF channel filled with 0.03 mg/mL FITC-Dextran aqueous solution (Fig. 5.4i), guaranteeing a conformal contact between the microgel and an inner wall of the PDMS channel.

To fabricate the MF channel for studies of lysis of fibrin microgels, the design of the second photomask was changed. Two symmetric side channels with the width of 300 \( \mu \text{m} \) and the length of 1.8 mm were placed upstream of the constriction at a distance of 100 \( \mu \text{m} \) from the end of the 2nd level region (Fig. 5.3D). The axisymmetric design facilities the mirror-image alignment of two identical PDMS replica, where the upper side channel of one PDMS replica is aligned with the lower side channel of the other replica. During lysis experiment, the upper side channel of the bonded PDMS MF channel was blocked, rendering the lower side channel mimicry of a needle for intravenous injection of thrombolytic agents.
Figure 5.3 Fabrication of multi-level microchannels. (A) Preparation of the 1st-layer pattern of the photoresist on silicon wafer using UV exposure of the photoresist with the first photomask. (B) Preparation of the 2nd-layer pattern of photoresist on silicon wafer by UV exposure (using the second photomask) of the photoresist sitting on top of the photoresist developed from (A). (C) Reflow of the double-layered photoresist pattern, followed by the preparation of PDMS replica from the photoresist and the alignment and bonding of two identical PDMS replica layers. (D) The second photomask used for the fabrication of the MF channel for lysis experiments. Two symmetrical side channels (width of 300 μm; length of 1.8 mm) were placed upstream of the constriction at a distance of 100 μm from the end of the 2nd level region.
5.5 Results and Discussions

5.5.1 Microgel properties

Fibrin hydrogel particles were prepared by MF emulsification of the mixture of aqueous solutions of fibrinogen, thrombin and Tris buffered saline (TBS) and subsequent fibrin gelation\cite{35} (Fig. 5.1A). Figure 5.1B and C shows a fluorescence microscopy image and the distribution of sizes, respectively, of fibrin microgels generated by the MF method. The microgels had a spherical shape (inset in Fig. 5.1B), a narrow size distribution with polydispersity not exceeding 5% and a uniform structure on the scale examined by confocal fluorescence microscopy of the microgels formed by fluorescein isothiocyanate (FITC)-labeled fibrinogen. The diameter of the microgels, $D_0$, was in the range from 150 to 220 $\mu$m, achieved by varying the flow rate of F-oil, $Q_c$, from 0.6 to 2.5 mL/h at the total flow rate, $Q_{tot}$, of the mixed aqueous phase of 0.3 mL/h (Fig. 5.1D). Notably, in vivo $>96\%$ of obstructive blood clots are smaller than 620 $\mu$m\cite{36}, that is, their dimensions were comparable to the microgel size used in our work.

Microgel composition was controlled by varying the ratio of flow rates of the aqueous streams carrying fibrinogen and thrombin, $Q_{fibrinogen}$ and $Q_{thrombin}$ respectively, at $Q_{tot} = Q_{fibrinogen} + Q_{thrombin} + Q_{buffer} = 0.3$ mL/h (the flow rate of the TBS stream, $Q_{buffer}$, was 0.1 mL/h). Notably, Ca$^{2+}$ ions were introduced into the microgels with a solution of thrombin in the amount of 10 mM. Figure 5.5A-C shows the composition and properties of the microgels prepared at varying $Q_{fibrinogen}/Q_{tot}$ ratio by the MF method. In this work, we assumed the concentrations of relative components (fibrinogen and thrombin) are the same for precursor droplets and microgels, and the difference between two states will be further addressed.
Figure 5.5 Composition, structure and properties of fibrin microgels. (A) Variation in the concentration of fibrinogen and thrombin in the precursor droplets, plotted as a function of the ratio $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$. (B) Permeability of fibrin gels with varying compositions prepared at varying $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$. Two red symbols are expected results, which have not been characterized experimentally. (C) Variation in the microgel stiffness, plotted as a function of the ratio of $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$. In (A-C) $Q_c = 0.8 \text{ mL/h}$, $Q_{\text{buffer}} = 0.1 \text{ mL/h}$, and $Q_{\text{tot}} = 0.3 \text{ mL/h}$. (D-F) SEM images of fibrin microgels obtained by gelation of the precursor droplets generated at $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$ ratios of 0.13 (D), 0.33 (E) and 0.53 (F), respectively. Insets show the fiber diameter, $D_f$, in respective fibrin gels. Scale bars are 500 nm.

Figure 5.5A shows that the variation in the concentrations of thrombin and fibrinogen in the precursor droplets, determined as described in section 5.3.1\cite{37}. The change in droplet composition correlated with the relative flow rate of the fibrinogen solution, $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$. Increase in $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$ resulted in a higher content of fibrinogen in the droplets and a corresponding decrease in the concentration of thrombin. Figure 5.5B shows the variation in permeability coefficient, $K_s$, of macroscopic fibrin gels with compositions determined by $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$, as in Fig. 5.5A. The value of $K_s$ increased from $1.9 \times 10^{-12}$ to $1.5 \times 10^{-10} \text{ cm}^2$, with the highest permeability corresponding to the gel composition corresponding to the lowest $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$. Note that two red symbols are expected results, which have not yet been characterized experimentally. Figure 5.5C shows that the stiffness of the fibrin microgels (studied by the micropipette aspiration method\cite{38}, as described in section 2.2.2.3) increased from 852 to 3601 Pa with an increasing $\frac{Q_{\text{fibrinogen}}}{Q_{\text{total}}}$. 

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\[ D_f = 180 \pm 38 \mu m \]
\[ D_f = 75 \pm 12 \mu m \]
\[ D_f = 10 \pm 3 \mu m \]
Later in the text, we focus on the microgels with the stiffness of 852, 1874, and 3601 Pa, and refer to them as soft microgels (SMs), medium rigidity microgels (MMs) and rigid microgels (RMs), respectively. These microgels were prepared at the lowest, the medium and the highest $Q_{\text{fibrinogen}}/Q_{\text{total}}$, respectively, and had fibrinogen content of 5.3, 22.8, and 37.9 mg/mL, respectively (Fig. 5.5A).

The structure of fibrin microgels was examined using scanning electron microscopy (SEM). Fig. 5.5D–F shows representative structures of SMs, MMs, and RMs. Thicker fibers with fewer branching points and larger pore size in SMs (Fig. 5.5D) strongly contrasted from thin fibers with a higher density branching and small pores in RMs, in agreement with earlier reports\cite{39,40}. The change in structure with microgel composition correlated with microgel properties in Figs. 5.5B and C: microgels with a larger pore size and thicker fibres had a higher permeability coefficient and lower stiffness, while short, thin and highly branched fibers formed rigid microgels with lower permeability. The structure of the microgels did not noticeably change following 30-day storage at 4 °C and closely resembled the structure of fibrin clots formed in vivo\cite{15}.

5.5.2 Occlusive behavior of fibrin microgels

A MF experimental setup similar to those in Chapter 3 and 4 was adopted, and the applied MF channel was fabricated as described in section 5.4. The MF platform mimicked thromboembolic effects, in which a thrombus formed in distant locations in the body travels with the blood stream into the brain, heart or lung to obstruct a blood vessel with the diameter significantly smaller than the thrombus size. For example, in embolic ischemic stroke (representing nearly 35% of all strokes\cite{41}) the thrombus completely obstructs a cerebral artery which supplies blood and nutrients to the cerebrum (Fig. 5.6A).

Figure 5.6B shows a schematic of the MF channel comprising (from left to right): a wide region (the channel-at-large), in which the spherical shape of the microgel remains unperturbed, a constriction with a diameter $d_c < D_0$, which is occluded by the fibrin microgel, and a wide channel-at-large (Fig. 5.4). In a typical experiment, a positive pressure difference, $\Delta P$, was applied to the microgel dispersion to introduce a microgel into a microchannel with the constriction with a diameter, $d_c = 65 \, \mu m$. The range of applied pressure differences was below 120 mmHg (0 to 16 kPa), similar to the cerebral perfusion pressure\cite{42}. 
Importantly, the constriction had a circular cross-section, thus providing conformal contact between the microgel and the walls of the microchannel (Fig. 5.4). Microfluidic experiments were performed for SMs, MMs and RMs. Figure 5.6C and D shows representative optical microscopy images of the non-deformed spherical fibrin microgel in the channel-at-large and an occlusive microgel localized in the constriction, respectively. The degree of microgel confinement in the constriction region was $2.3 \leq D_0/d_c \leq 4.2$, where $d_c$ the diameter of the constriction. The motion and the shape of the microgel in the microchannel were recorded and analyzed in a MATLAB program.

At a particular $\Delta P$ exceeding the translocation pressure difference $\Delta P > \Delta P_{tr}$, the microgel passed the constriction. Figure 5.6E shows the variation in the translocation pressure $\Delta P_{tr}$ for the SMs, MMs, and RMs subjected to a varying degree of confinement $D_0/d_c$ in the microchannel. For the same original microgel dimensions, $\Delta P_{tr}$ was the greatest for RMs, since it was harder to deform a microgel with a higher stiffness.

At a particular microgel stiffness, a greater $\Delta P_{tr}$ was required to force larger microgels into the constriction, owing to the stronger necessitated change in microgel size and shape. Notably, for SMs a very weak dependence existed between $D_0/d_c$ and $\Delta P_{tr}$.

Figure 5.6 Occlusive behavior of fibrin microgels. (A) Schematic of embolic ischemic stroke in vivo. An embolus formed in internal carotid artery, travels to the brain and completely blocks a cerebral artery,
resulting in cerebral ischemia. (B) Schematic of the MF channel comprised of a constriction and two channels-at-large at both sides of the constriction. The entrance angle, $\alpha$, to the constriction was 15°. (C, D) Non-deformed (C) and occlusive (D) fibrin microgels in the channel-at-large and in the constriction, respectively. Scale bars are 100 µm. (E) Variation in the translocation pressure $\Delta P_t$ with the degree of fibrin microgel confinement in the constriction. (F) Effect of the degree of confinement on the elongation strain of the microgel. (G) Variation in volume of occlusive microgels with their degree of confinement. The microgels were localized in the constriction for 20 min. In (E-G) black squares, red circles and blue triangles correspond to SMs, MMs and RMs, respectively.

Confinement of a fibrin microgel in the constriction resulted in a negative compressive strain $\gamma_c = (d_c - D_0)/D_0$, which led to a positive elongation strain $\gamma_e = (L-D_0)/D_0$ along the free, unconstrained microgel direction (Fig. 5.6F), where $L$ is the length of the occlusive microgel in the constriction. Surprisingly, for the degrees of confinements explored, the elongation strain, $\gamma_e = 1.12 \pm 0.03$, was not affected by the original microgel stiffness, structure or compression strain. A weak ~10% elongation of the compressed microgels was in marked difference with the behavior of occlusive agarose microgels with comparable stiffness, for which a similar degree of confinement in the MF channel led to ~30% elongation$^{[43]}$ ($D_0/ d_c = 2.5$). The strikingly weak elongation of fibrin microgels originated from a different mode of compression-induced deformation than that of the molecular gels. While molecular gels experience concentration-driven increase of osmotic pressure, until osmotic and elastic stresses in the unconstrained direction balance each other, for filamentous fibrin gel, compression may result in fiber buckling, rotation, and alignment in the direction perpendicular to the compression force$^{[21,22]}$.

Weak compression-induced stretching of fibrin microgels implied that change in structure and elongation-induced strain hardening, which were achieved at large elongation strains of macroscopic unconfined fibrin gels$^{[18]}$ should not be expected for occlusive fibrin clots. Instead, structural changes can be caused by compression and densification of fibrin clots due to their strong confinement-induced dehydration, accompanied by possible irreversible contacts of fibrin filaments$^{[22]}$.

The equilibrium volume, $V$, of the microgels lodged in the constriction greatly reduced in comparison with their original unperturbed volume, $V_0$, (Fig. 5.6G), due to the squeezing of water from the microgel interior. For the same degree of confinement, larger microgels experienced stronger shrinkage. For example, for microgels with $D_0$ of 150 and 265 µm, ~78% and ~95% of water was expelled from the microgel, respectively. Importantly, for the same degree of microgel confinement in the constriction, the relative
change in microgel volume was not affected by a ~ 7-fold change in their stiffness, implying that at a particular confinement $D_0/d_c$, the microgels exhibited the same reduction in $V/V_0$, the effect originating from almost constant elongation strain.

### 5.5.3 Microgel relaxation

In the constriction, occlusive fibrin microgels adopted a nearly cylindrical shape (Fig. 5.7A) and exhibited strong birefringence, as follows from the polarized optical microscopy (POM) image in Fig. 5.7A'. While original, non-confined spherical microgels showed no birefringence (Fig. 5.8F), strong birefringence suggested the alignment of fibrin fibers along the long axis of the occlusive microgel. Interestingly, microgel length in the POM images was smaller than in the bright-field images, which signified a stronger extent of fiber alignment in the central region of the microgel, experiencing a larger compressive strain.

Following insertion and maintenance of the fibrin microgel in the constriction, it was evacuated in the channel-at-large by applying a negative pressure. For the released microgel, we explored the relaxation of its size and shape, as well as the decay in birefringence, that is, fiber alignment. The recovery of these properties did not change with 1 - 4 h confinement of the microgel in the constriction; thus the relaxation experiments were conducted for the microgels lodged in the constriction for 1 h. In Fig. 5.7B, C, B', and C' representative bright field optical microscopy and POM images show a gradual, yet not complete recovery of these properties. After 7 h relaxation (Fig. 5.7C and C') and even after up to 24 h equilibration time (Fig. 5.8E, J), the released microgels exhibited a non-spherical shape, a smaller than original size and residual birefringence indicating the remaining unidirectional fiber alignment.

Quantitatively, the relaxation of microgel shape, size and birefringence is shown in Fig. 5.7D-I, where the notations $t$ and 0 correspond to the microgels released from the constriction after time $t$ and to the microgel lodged in the constriction at $t = 0$, respectively. The recovery of the microgel shape was characterized by measuring the temporal decrease in its aspect ratio, $AR(t)/AR_0$, where $AR$ is the ratio of the lengths of the long and short microgel axes. The relaxation of the microgel size due to its re-swelling was characterized by measuring its area as $[1/A(t)]/[1/A_0]$. Since the dimensions of the channel-at-large were larger than the microgel size, the rate of area relaxation was viewed as a measure of the rate of volume relaxation of the
microgel. The relaxation of fiber orientation in the microgels was characterized by measuring the decay in microgel birefringence\(^{44}\), that is, the reduction in its normalized average grey scale as \(I(t)/I_0\), where \(I\) is the average grey scale of the entire microgel.

Figures 5.7D-I show several trends in fibrin microgel recovery after confinement. All microgels showed fast (within ~ 15 sec) partial recovery, with SMs and RMs recovering ~ 10 and 30 - 50% of their properties, respectively. The fast relaxation stage was followed by the slower recovery, in which within ~ 3 h RMs reached 60 - 90 % recovery, while SMs and MMs gradually recovered up to 30 and 40 % of their properties. These trends agreed with the results of rheological studies of fibrin clots\(^{45,46}\), in which clots comprised of thicker fibers displayed slow recovery, due to the stronger lateral aggregation of protofibrils\(^{47}\). With an increasing degree of confinement \(D_0/d_c\), the RMs recovered faster. For these microgels, fiber alignment characterized by the reduction of birefringence occurred faster than the recovery of microgel size and shape.

Figure 5.7 Relaxation of fibrin microgels following their release from the constriction (A-C, A’-C’). Bright field (A-C) and POM (A’-C’) images of fibrin microgel maintained in the constriction for 1 h at the degree of confinement \(D_0/d_c = 3.64\) (A and A’), and 17 sec (B and B’) and 7 h (C and C’) after removing it from the constriction. Scale bars are 100 \(\mu\)m. (D-I) Relaxation of microgel normalized average grey scale (D, E), aspect ratio (F, G) and normalized area (H, I) confined to a degree of confinement \(D_0/d_c = 2.89 \pm 0.29\) (D, F, and H) and \(D_0/d_c = 3.64 \pm 0.04\) (E, G, and I). In each graph, SM (hollow symbols), MM (half-solid symbols), and RM (solid symbols) was plotted as a function of time after microgel release from the constriction, respectively.
5.5.4 Flow of water through the occlusive microgel

At pressure difference $\Delta P < \Delta P_{tr}$, a fibrin microgel clogged the constriction entrance, with portions of its body localized in the tapered zone and in the constriction (Fig. 5.9A). The position of the microgel center, $X_c$, was approximated as $X_c = (X_b + X_f)/2$, where $X_b$ and $X_f$ are the positions of the back and front edges of the microgel, respectively, with respect to the beginning of the constriction, $X_0$. We performed fluorescence recovery after photobleaching experiments to determine the rate of water flow, $Q$, through the trapped microgels, since convection-driven flow can contribute to the rate of lysis of occlusive clots. The method to measure $Q$ was as described in section 4.3 in Chapter 4. Prior to the experiments, microgels were suspended in 0.03 mg/mL TBS of dextran molecules labeled with fluorescein isothiocyanate and diameter of 12.0 nm\[48\]. Since occlusive microgels were in conformal contact with microchannel walls, no leakage occurred and the flow of water did not exceed $1.2 \times 10^{-5}$ mL/h (or 3200 $\mu$m$^3$/s).

Figures 5.9B-D show a non-monotonic variation of the flow rate, $Q$, with the microgel position in the microchannel for occlusive SMs, MMs and RMs with different original dimensions. For all the microgels, an original increase in water flow rate at low $\Delta P$ (or weak confinement in the constriction) was followed...
by the reduction in $Q$ at the higher pressure difference corresponding to stronger microgel confinement with a higher fraction of water excluded and a smaller pore size. For the microgels with the same stiffness, the maximum in $Q$ on the bell-shaped curve shifted toward larger $X_c$ values with an increasing degree of microgel confinement. In other words, for occlusive microgels with a larger original diameter a higher $\Delta P$ was required to achieve the maximum water flow rate. For the microgels with similar original diameter, $D_0$, the peak position shifted to a smaller $X_c$ with increasing microgel stiffness, because a larger force was required to insert the microgel to a similar position in the microchannel.

Figure 5.9 Variation in the flow rate of the liquid through occlusive fibrin microgels. (A) Optical microscopy image of a RM ($D_0 = 200 \, \mu m$) trapped at the entrance of the constriction. Vertical red and green dashed lines give outline the position of the back edge, $X_b$, and front edge, $X_f$, of the microgel, respectively, with respect to the beginning of the constriction, $X_0$. Scale bar is 100 $\mu m$. Experimentally measured volumetric flow rate of liquid through confined SMs (B), MMs (C) and RMs (D), plotted as a function of the position of microgel center ($X_c$). Insets show the unperturbed size the confined microgel.

5.5.5 Lysis

Lysis experiments were conducted for the SMs, MMs, and RMs with a varying initial diameter. The microgels were incubated for 2 h in the 1.07 $\mu M$ solution of the native glutamic acid-plasminogen (Glu-Plg) to ensure diffusion of Glu-Plg into the microgel interior. The microgel was positioned in the tapered region of the microchannel at $\Delta P = 700 \, \text{Pa}$, with part of it localized at the constriction entrance (Fig. 5.10A). A mixed solution of 7.4 nM AlexaFluor633-labelled t-PA, a plasminogen activator, and 0.1 mg/mL FITC-Dextran (MW = 70 kDa) in TBS was introduced at a flow rate of 0.2 mL/h to a side channel orthogonally to the long axis of the main microchannel, thus mimicking intravenous injection of t-PA in a blood stream. The mixed solution reached the left back edge of the occlusive microgel by convection and diffusion. The
shape and the area of the confined microgel were monitored over 7 h with intervals of 30 sec from the moment when the dissolution of the microgel was observed (taken as $\tau = 0$).

When the mixed solution containing t-PA reached the microgel, the back microgel edge became blurry, due to the onset in gel digestion (compare Fig. 5.10B at $\tau = 0$ min and $\tau = 6$ min). Over longer lysis time (up to $\tau = 160$ min), the microgel gradually shrank and its back edge moved towards the constriction (the position of the front edge of the microgel was invariant). At $\tau = 161$ min, a shrunk microgel was evacuated from the microchannel with the liquid flow and the microchannel was recanalized with the liquid (Fig. 5.11).

In the course of fibrinolysis experiments, based on Fig. 5.9 and Fig. 5.11, both t-PA and FITC-labeled dextran solutions permeated the microgel. However, t-PA attached to the surface of fibrin and accumulated in the interior of the shrinking microgel, as suggested by the gradually increasing intensity of pink color in the microgel (Fig. 5.10B). In contrast, the solution of FITC-labeled dextran moved through the microgel without accumulation, with the rate controlled by applied pressure difference, as shown in Fig. 5.9B-D. With progressive microgel digestion, the flow of FITC-labeled dextran through the microgel gradually enhanced, until the microchannel was completely recanalized (Fig. 5.12). Such enhancement was stronger for smaller microgels, in correlation with a stronger convection-driven liquid flow through smaller occlusive microgels, as shown in Fig. 5.9.
Figure 5.10 Lysis of fibrin microgels. (A) Schematic of the lysis experiment. A mixed solution of 7.4 nM AlexaFluor633-labeled t-PA and 0.1 mg/mL FITC-Dextran (MW = 70 kDa) in TBS is injected at a flow rate of 0.2 mL/h via a side channel placed 148 μm from the occlusive microgel. During injection and in the lysis experiments, the microgel is maintained at ΔP = 700 Pa. (B) Representative merged multi-channel microscopy images of occlusive MM (D₀ = 200 μm), following its digestion. Blue dashed lines show the back and front edges of the microgel at τ = 0. Green and pink color correspond to FITC-Dextran (70 kDa) and AlexaFluor633-labeled t-PA, respectively. Scale bar is 100 μm. (C) Relative change in volume of occlusive RM, MM or SM with lysis time τ. D₀ = 197.1 ± 3.3 μm. (D) Relative change in volume of occlusive RMs with varying initial dimensions, plotted as a function of lysis time.

Figure 5.11 Merged-channel and individual channel images of a microgel (D₀ = 200 μm) during lysis, as in Fig. 5.10. Scale bars are 100 μm. Red color originates from AlexaFluor633-labelled t-PA, and green color originates from FITC-Dextran.
Figure 5.12 Variation in the relative FITC intensity of a region of the microchannel downstream of the occlusive microgel with lysis time. Top row: representative FITC-channel images captured at various time points. Bottom row: concentration of FITC-Dextran within a particular channel portion downstream of the occlusive microgel as a function of time. Inset shows enlarged plot. Red symbols correspond to the images shown in the top row. The selected microchannel region with dimensions 120 \( \mu \text{m} \times 60 \mu \text{m} \) was 300 \( \mu \text{m} \) downstream of the beginning of the constriction. The relative FITC intensity was measured using NIS Element software, and represented the difference with the background. Scale bars are 100 \( \mu \text{m} \).

Microgel digestion rate was examined for RMs, MMs, and SMs with a diameter \( D_0 = 197.1 \pm 3.3 \mu \text{m} \), each placed at the same position in the microchannel (Fig. 5.10C). Microgel lysis was characterized as the variation of \( V(\tau)/V_0 \) vs. \( \tau \), where \( V(\tau) \) and \( V_0 \) represent the volumes of the occlusive microgel at lysis time \( \tau \) and \( \tau = 0 \), respectively. The rate of fibrinolysis decreased from SM to MM to RM. For example, at \( \tau = 78 \) min, the SM shrank to 4\% of its original volume and passed the constriction, while the MM and RM retained 16 and 20\% of their volumes, respectively, and obstructed the microchannel. These results agreed with earlier findings on an easier digestion of thicker than thinner fibrin fibers\(^{[27]}\), due to the more efficient plasmin action on tightly packed fibrin monomers within a cross-section of a thick fiber, in comparison with slower plasmin diffusion through the pores of the network of thin fibers\(^{[8]}\).

The dependence of microgel lysis on its degree confinement was studied for the microgels with varying original diameters \( D_0 \) and stiffness \( S = 3500 \) Pa (Fig. 5.10D). Longer time was required to evacuate larger fibrin clots from the microchannel, indicating that lysis rate depended on the compressive strain of the occlusive microgel. With lysis progression from the back edge of the clot, microgel confinement became stronger, due to reducing microchannel diameter, thereby leading to the continuously increasing strain (in assumption that t-PA was introduced in excess). Therefore, at longer lysis time \( \tau \), the graphs in Figs. 5.10C and D became more non-linear.

5.6 Conclusions

In this chapter, we developed a MF platform for studies of thromboembolism and fibrinolysis of occlusive blood clots. We used uniformly-sized spherical fibrin microgels with varying compositions and structures as artificial fibrin clots. A MF channel with a narrow circular cross-section constriction was used as a biomimetic model of a blood vessel. When subjected to the same strain in the constriction, soft microgels...
with thick fibers (generated at a low fibrinogen-to-fibrin ratio) exhibited a stronger irreversible deformation compared to the rigid microgels with a dense branched network structure (prepared at a high fibrinogen-to-fibrin ratio). In addition, the former microgels were more liquid-permeable and underwent faster fibrinolysis. Importantly, upon bi-axial compression in the microchannel very weak elongation was observed for occlusive fibrin microgels, independently of their dimensions and composition. This observation contrasts with current expectations that uniaxial elongation dominates strain-induced structure and properties of blood clots \textit{in vivo}, and indicates the importance of \textit{in vitro} studies of thromboembolism. Flow of liquid through occlusive clots was non-linearly dependent on the clot position in the MF channel, and was enhanced during fibrinolysis. The rate of lysis decreased with stiffness and dimensions of the microgels. The proposed platform paves the way for fundamental studies of the complicated relationship between the structure, viscoelasticity, and fibrinolytic resistance of blood clots, and has direct implications for the development of effective therapeutic treatment of thromboembolism \textit{in vivo}. 
References

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Chapter 6
Fabrication of in vitro microvascular system for study of pulmonary embolism

6 Fabrication of in vitro microvascular system for study of pulmonary embolism

6.1 Introduction

It is well known that blood vessel geometry, size and mechanical properties, as well as interactions of blood components with the endothelial surface, greatly influence the flow of blood through the vasculature. This, in turn, affects several physiological processes such as endothelial cell (EC) differentiation, plaque formation and embolization. To perform a systematic study of these phenomena, and also to engineer tissues with blood vessels, the research community has, over the past two decades, attempted to produce tunable, in vitro replicas of the three-dimensional (3D) microvascular architecture. The ultimate aim is to have a microfabrication technology that can provide a vascular microcirculation system comprised of arterioles (250 μm > diameter ≥ 8 μm), capillaries (diameter < 8 μm), and venules (250 μm > diameter ≥ 8 μm) that replicates the complex structure of the blood vessel network in vivo[1], with the appropriate mechanical properties and EC linings in the various vessels. While significant progress has been made towards this aim over the past two decades due to the emergence of various microfabrication technologies, the research community is still far away from the holy grail.

The most widely accepted method for the fabrication of biomimetic microvessels is based on soft lithography in different polymeric substrates[2-5]. Since the advent of soft lithography[6,7], poly(dimethylsiloxane) (PDMS) based microfluidic channels have been more widely used for biological and chemical applications compared with other polymer materials[2-5]. PDMS devices are cost-efficient, optically transparent, oxygen-permeable and non-cytotoxic. Furthermore, the ability to replicate complex geometries of the micro-cardiovascular system and to precisely tune the dimensions of microchannels have made the soft lithography method very beneficial for cell studies[8]. However, the rectangular shape of the cross-section of PDMS channels[9] makes it challenging to establish fully endothelialized microenvironments. The inhomogeneous deposition of cells in the sharply varying regions of the geometries (e.g., corners)[10] leads to strong variations in physiology of the cultured cells[11]. Hydrodynamic
stresses can vary strongly in these regions as well \cite{12,13}, and thus can impact on flow-based studies significantly\cite{14-16}. The cross-sectional geometry of the artery also affects the shear stress imposed on ECs\cite{11,17}, potentially influencing such various processes as cell differentiation\cite{18}, tight-junction formation\cite{19}, gene expression\cite{20,21}, and response to inflammation\cite{22,23}. Therefore, the flow geometry needs to be as close as possible to the geometry of \textit{in vivo} microvessels because of its essential role in the formation and maturation of vessels. Another reason for requiring a circular cross-section is the accurate modeling of the embolization of small microchannels by soft particles such as blood clots. When soft particles are trapped at constrictions with rectangular cross-sections under an imposed pressure difference, a parasitic leakage flow occurs through the corners of the cross-section, which strongly affects the measurements of the pressure-induced translocation characteristics of the soft particle. This leakage is absent when the cross-section is circular.

Unlike the microchannels fabricated from poly(methyl methacrylate), polycarbonate and other rigid polymeric substrates by micromilling or embossing\cite{3,4,24,25}, it is possible to modify the cross-section of a straight PDMS microchannel from a rectangular shape to a circular shape by directly molding the circular channels inside of PDMS\cite{26,27}. This approach is suitable for a single straight cylindrical microchannel, and for a channel with a single constriction\cite{28}. Other options exist for producing microchannels with circular PDMS cross-sections. Metal wires\cite{29}, stainless steel needles\cite{30}, liquid PDMS channels re-modified by pressurized air streams\cite{26,27} or inkjet printing\cite{31} have been reported to directly create a single straight cylindrical channel in PDMS. However, all of the above methods suffer from the same drawback: the inability to reproducibly fabricate a more complicated network of channels with circular cross-sections and well-controlled channel dimensions. The vessels found in mammalian cardiovascular and respiratory systems are usually arranged in hierarchical structures and a distinctive feature of this arrangement is their multi-stage division or bifurcation\cite{32}. At each generation, the characteristic dimension of the vascular segments will generally become smaller, in both length and diameter. However, many current fabrication processes cannot create multi-depth channels without multistage operations to align and expose the mask.

The issue of hierarchical architectures with multiple generations of microchannel sizes can be approached using conventional lithography and molding approaches\cite{33-36}. But apart from the problems with the rectangular cross section discussed above, the microchannels in every branch generation inherently retain identical channel heights. This renders the shear rates highly non-uniform, because large aspect ratio
channels exhibit much stronger velocity gradients in the thin direction than the longer direction. This anisotropy in the shear stresses compared to the flow through an axisymmetric circular cross-section is unfavourable for experiments. One way of avoiding rectangular cross-section is to use sacrificial 3D templates obtained from either 3D printing\cite{37-39} or omnidirectional printing\cite{40}; however the 3D printing resolution (ca. 200 μm)\cite{41} and restrictions on the material choice is the current limitation.

There is a severe need, therefore, for a simple, cost-efficient method for fabricating a microchannel network with multi-level branching channels, with multiple depths at different generations, and with circular cross-sections in the straight sections and smoothly varying cross-sections at the branching points. We report in this chapter a method for generating such a microchannel network in PDMS by using a combination of photolithography, photoresist “reflow” techniques and soft lithography. The method is inspired by several prior publications\cite{42-45} that demonstrate the production of a microchannel master with a semicircular cross-section using thermally-induced flow of the polymer above its glass transition temperature. Two PDMS molds with semicircular grooves produced from the same master yield a microchannel with a circular cross-section when bonded to each other. The method can also be used to fabricate a microchannel network, but all branches in the resulting network can have only one diameter or very similar diameters due to a limitation of the method. In this work, we have implemented a critical modification to this technique that overcomes this limitation and allows the production of different diameters of nearly circular microchannels at the different branching levels of the network.

This chapter is organized as follows. Section 2 discusses materials employed in the experiments, the basis of the fabrication method and the fabrication procedure. Section 3 discusses the experimental results of the characteristics of the microchannel network depending on the fabrication conditions. We also demonstrate the use of the microchannel network to study the blockage of daughter microchannels by fibrin clots and the subsequent redistribution of liquid through other branches of the network. We summarize our findings in Section 4.
6.2 Methodology, materials and methods

6.2.1 Methodology

The reflow or the photoresist melting technique developed by Wang et al\textsuperscript{[42]} and improved by others\textsuperscript{[43–45]} relies on the idea that, in the absence of external forces, a given volume of liquid in contact with air rearranges its shape so as to minimize its interfacial energy. Consider a layer of AZ40XT photoresist deposited on a substrate using traditional photolithographic techniques in the form of straight channel with a rectangular cross-section of width $W$ and height $H$ (see Fig. 6.1). When the temperature of the photoresist is raised to a temperature $T_m$ beyond the glass transition temperature ($T_g$) of the polymer for a specified period of time $t_R$, the polymer softens, capillary forces cause the sharp curvatures in the initially rectangular cross-section of the polymer-air interface to relax to a circular sector of radius $R$ and contact angle $\theta$. By appropriate selection of the initial dimensions of the photoresist layer and the operating temperature ($T_m$), and by ensuring that the duration of melting ($t_R$) exceeds the capillary time scale ($t_c$), the contact angle $\theta$ can be tuned to a $90^\circ$, i.e., the circular sector becomes a semicircle. The capillary time scale $t_c$ is defined as $\frac{\mu W^4}{\gamma H^3}$, where $\mu$ and $\gamma$ are the viscosity and interfacial tension of the polymer at the temperature $T_m$. The resulting master can be employed to produce two complementary PDMS molds having semicircular grooves as channels, and the bonding of these molds produces a channel with circular cross-section.

![Figure 6.1 Schematic of the reflow process of AZ40XT photoresist in cross-sectional view on a silicon wafer.](image)

If the evaporation-induced loss of solvent from the photoresist during the reflow process leads to the final cross-section area being a fraction $f$ of the initial area $HW$, then simple geometrical arguments show that the contact angle is related to the geometrical parameters by the non-linear relationship,
If the width of the polymer phase in contact with the substrate changes by a factor \( c \) relative to original width \( W \), then \( cW = 2R \sin \theta \), and therefore

\[
\frac{fHW}{c^2W} = \frac{(\theta - \sin \theta \cos \theta)}{4\sin^2 \theta}.
\]  

(6.2)

Figure 6.2 A plot of the contact angle as a function of \( \frac{f}{c^2} \times \frac{H}{W} \), as predicted by Eq. 6.2.

The relationship between contact angle and \( \frac{f}{c^2} \times \frac{H}{W} \), as deduced from the above equation, is shown in Fig. 6.2. In many of our experiments, the width of the photoresist in contact with the substrate changes very weakly (< 10%), due to the negligible gravitational effects (the Bond numbers for the dimensions involved are weak), and also presumably due to the contact line resistances. Also, if the cross-sectional area is modified insignificantly due to the melting of the photoresist, then the x-axis of Fig. 6.2 (the LHS of the above equation) reduces to the aspect ratio \( H/W \). Figure 6.2 suggests that, to get a contact angle of 90°, the initial height \( H \) of the photoresist needs to be a factor of about 0.4 times the initial width \( W \) of the photoresist layer. Larger diameters of the final circular channel require wider and taller photoresist layers at the outset.

The problem with the current protocol of the reflow technique, as mentioned in the introduction, is that one cannot produce a network of semicircular structures of different sizes on the same master. Since the initial master with the rectangular cross-section is produced by spinning a layer of photoresist material, the height of this layer,
$H$, is constant everywhere on the substrate. The width $W$ of the rectangular cross-sections can be selected to be different in different parts of the network using photomasks, but the height $H$ of the entire network of the master is fixed by thickness of the photoresist layer. Thus, there is no independent control of the contact angle when different diameters are required on the same master, as has been realized in previous attempts at making more realistic replications of vascular networks\cite{33-36}.

To rectify this deficiency, we introduce an additional step in the fabrication of the master. The schematic of this procedure is shown in Fig. 6.3. The procedure begins with the production of a layer of SU-8 photoresist in the regions where a larger diameter is desired. The cross-section of this SU-8 layer is rectangular with a width $W_0$ and height $H_0$. This is followed by a layer of the AZ40XT photoresist over the existing SU-8 features (Fig. 6.3A), and also over other regions where a smaller diameter is desired (Fig. 6.3B). If the height $H_0$ of the SU-8 layer is large enough, then the height of the spun AZ40XT layer in the regions with the pre-existing SU-8 layer is greater than that in the regions where SU-8 is absent. The temperature is then raised to a value where AZ40XT melts, but SU-8 does not. Since a larger height of the AZ40XT photoresist is available in the regions where a larger diameter is desired, and if approximately the same initial $H/W$ ($H_1/W_1$ in Fig. 6.3A; $H_2/W_2$ in Fig. 6.3B) of about 0.4 can be maintained, melting will result in two different diameters in the two different regions of the master. This idea is tested in this chapter for different widths and heights of the SU-8 features.

![Schematic of reflow of AZ40XT photoresist into semi-circular cross-section](image)

**Figure 6.3 Schematic of reflow of AZ40XT photoresist into semi-circular cross-section.** (A) A region with wider dimension, an additional layer of SU-8 negative photoresist was formed before spin-coating of AZ40XT, resulting in a thicker photoresist in comparison with (B) narrower region, where AZ40XT reflows into semi-circular cross-section without insertion of SU-8 below.
6.2.2 Materials

All solvents were obtained from Fisher Scientific unless otherwise specified. Silicon wafers were purchased from Wafer World, Inc. (West Palm Beach, FL). SU-8 2025 photoresist and SU-8 developer were from MicroChem Corp. (Newton, MA). AZ40XT photoresist and MIF-300 developer were from Integrated Micro Materials (Argyle, TX). Dow Corning Sylgard 184 Silicone elastomer kit which contained poly(dimethylsiloxane) (PDMS) prepolymer and crosslinker was from Ellsworth Adhesives Canada (Burlington, ON). Bovine fibrinogen, Bovine thrombin, Tris buffered saline (TBS), Span 80, hexane, Tween 20, Hexamethyldisiloxane (HMDS, ≥ 98%), trichloro(1H,1H,2H,2H-perfluorooctyl)silane (97%), and fluorescein isothiocyanate-dextran (FITC-Dextran) (MW = 70 kDa) were from Sigma-Aldrich Canada (Oakville, ON). Fluorinated oil (F-oil) HFE-7500 3M Novec was from 3M. Mono-sized cross-linked Poly(methyl methacrylate) (PMMA) microbeads (6 μm) was from Microbeads AS (Skedsmokorset, Norway). Monodispersed fluorescent polystyrene microspheres (1 μm, 2.5% aqueous suspension) was from Polysciences, Inc. (Warrington, PA). All chemicals were used as received without purification. The triblock copolymer surfactant of perfluoropolyethers and a poly(ethylene oxide) polypropylene copolymer [PFPE-P(EO-PO)-PFPE] was synthesized as described previously.

6.2.3 Microchannel design

The design of a multiple-branch channel network is based on Murray’s law[46], which states that the cube of radius of a parent branch equals the sum of the cubes of the radii of the daughters[47]. Figure 6.4A shows the three-generation design of branched microchannel with the largest channel (first generation) set as 200 μm, and the diameters of its lower branch channels were calculated based on Murray’s law (i.e., 158 μm and 126 μm for second and third generation, respectively). The angles at the bifurcations were designed as 45°. Double UV exposure (Figs. 6.4B and C) was designed to prepare hybrid structure including SU-8 and AZ40XT in the final template, therefore, the pattern of the photomask of the second exposure is exactly as the microchannel design discussed above. For the pattern of the photomask of the first exposure, microchannel without second or third generation was designed, and the width of the remaining first generation microchannel was designed to be smaller than 200 μm (details discussed in later section).
Figure 6.4 Schematics of microfabrication procedure. (A) Complete pattern of second photomask, where the width of the first, second and third generation is 200 μm, 158 μm, 126 μm, respectively. (B) Preparation of first layer of microstructure of SU-8 2025 photoresist on silicon wafer by UV exposure of the photoresist with the first photomask. (C) Preparation of second layer of microstructure of AZ40XT photoresist on top of the developed first-layered structure with the second photomask. (D) Reflow of the second-layered photoresist, followed by the preparation of PDMS replica from the photoresist and the alignment and bonding of two identical PDMS replica layers. Pattern of photoresist in the schematics is simplified into single channel from branched microchannel.

6.2.4 Fabrication of master molds with semi-circular cross-section

The first step in the fabrication process is to generate an SU-8 2025 negative photoresist master having a rectangular cross-section by using the first photomask (Fig. 6.4B). Two designs of first photomask with different width (100 μm and 150 μm) were used to UV expose the layer of negative photoresist with various thickness (20 μm, 35 μm, and 65 μm) from varying spin coating rates (4000 rpm, 2000 rpm, and 1000 rpm). After development, SU-8 structure with different width and height in cross-section shape were obtained on the silicon wafer.

The second step is to generate rectangular cross-sectional shapes of the AZ40XT positive photoresist by using the second photomask (Fig. 6.4C). The Si wafer with SU-8 features was treated with HMDS (Hexamethyldisiloxane) at 75 °C for 40 min to render the surface hydrophobic. The positive photoresist with
various thickness was spin-coated on the Si wafer at varying spin coating rates (400, 600, 800 and 1000 rpm) for 30 sec and a high rate (2500 rpm) for 2 sec to minimize edge bead, followed by the solvent evaporation for 1 h. A three-stage baking step was then proceeded at 70 °C for 5 min, 90 °C for 5 min, and 115 °C for 5 min. The positive photoresist was exposed to UV light (70 sec, 14 mW/cm², 365 nm) after aligning the photomask with the already formed SU-8 structure (Fig. 6.4C), post-baked at 100 °C for 30 sec, and developed with MIF-300 developer using puddle development process with a total development time of 5 min. During the puddle mode, MIF-300 developer was poured onto the stationary wafer with photoresist that was allowed to sit motionless for 1 min, and the wafer was then spin rinsed and dried. A total of 5 cycles of the puddle procedure above was applied to achieve a complete development of the positive photoresist which sat on top of the SU-8 structure.

After development, AZ40XT photoresist was reflowed by heating with varying temperature and period in the third step (Fig. 6.4D). The reflowed structure was then treated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane at 75 °C for 1 h. PDMS precursor and PDMS curing agent, mixed at a volume ratio of 5:1, were poured into the convex half-circular photoresist on a Si wafer, and cured in an oven at 73 °C for 1 h. The cured PDMS was then carefully peeled off from the Si wafer, and the geometry of the PDMS replica was characterized by the contact angle (θ) as defined in Fig. 6.1.

To align and bond two identical PDMS layers, the featured surface was treated for 10 sec for each layer with a hand-held BD20-AC corona treater (Electro-Technic Products Inc., Chicago, IL), followed by the addition of 10 μL filtered ethanol in between of two layers, and the careful alignment of the two corona-activated layers under the microscope with 4X objective lens. The position of two aligned layers was maintained until the ethanol completely evaporated via a 250W incandescent heating lamp (NOMA Corporation, Canada) held on top of the microscope. The dried two layers were then pressed together and left undisturbed in a 115 °C oven overnight to obtain a permanent bonding.

6.2.5 Fibrin gel preparation

Please refer to the Section 5.2.2 “Microfluidic preparation of microgels” for more details. Briefly, fibrin microgels with a diameter \( D_0 \) in the range \( 190 < D_0 < 220 \ \mu\text{m} \) and stiffness of 3.5 kPa were prepared by the
microfluidic (MF) emulsification of the mixture of fibrinogen, thrombin and Tris-buffer solutions and subsequent gelation of fibrinogen. Complete fibrin gelation was achieved by incubating microgel dispersion collected at the exit from the MF device for 1 h in a 37 °C water bath. The fibrin microgels were transferred from F-oil into Tris-buffer containing 0.02 wt% of surfactant Tween 20 by dispersion following centrifugation and the separation of the supernatant.

6.2.6 Microfluidic setup for occlusion studies

The MF experimental setup was described in detail in Section of 2.3.2.1 “Entrance studies”. In short, the pressure drop across the MF channel was controlled by varying the difference in the levels of buffer suspension between the upstream and downstream reservoirs. The motion of the microgels in the branched microchannels was recorded with a high-speed camera (Phantom v-Series) at 4000 frame per second (fps) and analyzed by Phantom Camera Control application and Image Pro Plus. All experiments were performed at room temperature.

6.2.7 Flow rate measurement in microfluidic channels

Tris-buffer solution containing 0.02 wt% Tween 20 and 0.007 wt% fluorescent beads filled in the upstream reservoir. Tris-buffer suspension of 0.15 wt% PMMA beads containing 0.02 wt% Tween 20 filled a syringe which attached to the upstream reservoir with a three-way connector. After injecting PMMA bead suspension into the system, the MF system was closed and left undisturbed for 5 min until the 6 μm-PMMA beads settled at the bottom of the microchannel due to gravity. Careful focus of the settled PMMA beads was performed within the channel, and the focus plane was moved manually upwards by a distance equal to the radius of the microchannel to locate the center plane of the microchannel. Following the location of focus plane, a syringe containing the microgel suspension with 0.007 wt% fluorescent beads substituted the syringe with PMMA bead suspension and attached the upstream reservoir with a three-way connector. The velocity of the fluid at the center line was measured by tracking the motion of the PMMA beads with a high-speed camera. The average fluid velocity through the circular channel is, simply, one half of the centerline value, and the flow rate is the average velocity multiplied by the cross-sectional area.
6.3 Results

6.3.1 Variation of reflowed structure with varying photoresist thickness

Figures 6.5A-C show bright field optical microscopy images of the semi-circular cross-sectional PDMS replica of three generations from reflowed geometry of the photoresist, achieved at 100 μm × 35 μm cross-section of SU-8 structure, 67.9 ± 1.0 μm thick of positive photoresist, reflow at 130 °C for 70 sec. The contact angle of the three generation branches is 85°, 96°, and 90°, respectively, in Fig. 6.5A-C. The cross-sectional images of three generations of aligned and bonded PDMS microchannel from Fig. 6.5A-C are shown in Fig. 6.5D-F, respectively.

To characterize the three-dimensional (3D) feature of the PDMS microchannel, 0.03 mg/mL FITC-Dextran aqueous solution was filled in the channel and a Confocal Laser Scanning Microscopy (CLSM) z-stack scan of the channel was executed. A tilted angle view of the 3D reconstructed microchannel from second to third generation, illustrating the smoothness of the profile of the microchannel (Fig. 6.5G).

![Figure 6.5 Characterization of reflowed branched microchannel.](image)

Figure 6.5 Characterization of reflowed branched microchannel. (A-C) Optical microscopic images of cross-sectional view of PDMS replica in first (A) second (B) and third (C) generation of branched microchannel. (D-F) Optical microscopic images of cross-sectional view of aligned and bonded PDMS microchannel in first (D), second (E) and third (F) generation of branched microchannel. (G) A tilted angle view of the 3D reconstructed microchannel from second to third generation. The 3D feature was obtained from a z-stack of Confocal Laser Scanning Microscopy (CLSM) images, scanned at a step of 5 μm for an entire z range of 200 μm. Assembled microchannel was filled with 0.03 mg/mL FITC-Dextran aqueous solution. Scale bar is 100 μm and applies to all images.

Figure 6.6A shows the variation in the contact angle of the three generations of branched microchannel, plotted as a function of the thickness of the spin-coated AZ40XT when the reflow condition was set as 130 °C for 70 sec. The thickness of the positive photoresist was controlled by varying the spin coating rate (1000, 800, 600, and 400 rpm for 55, 65, 75, and 85 μm, respectively). Typical images of three generation cross-sections of channel for thickness of 55 and 85 μm are shown in Fig. 6.6D-G. The contact angle increased with generation.
order for each thickness. The contact angle for each generation also increased with thickness of photoresist film, although this variation was most sensitive for the 1st generation. Figure 6.7 shows the variation of the contact angle with $H/W$ for this data, along with the prediction from Eq 6.2 with $f = 1$ and $c = 1$ (solid blue line). While the $H = 55 \, \mu m$ data (crosses) appear to be well described by the model, the 65, 75 and 85 $\mu m$ data deviated systematically and progressively below from the model prediction. This is likely due to the slight increase in the width ($c > 1$) and the increased loss of solvent from ($f < 1$), the neglect of both of which lead to overprediction of the contact angle for a given $H/W$. An examination of Fig. 6.7 shows that for $H = 65 \, \mu m$ (reflow at 130 °C for 70 sec), the second and third generation showed contact angles that were only slightly below and slightly above 90° respectively, but the contact angle for the first generation was 72°, which is much below 90°.

Figure 6.6 Variation of reflowed structure with varying photoresist thickness. (A) Effect of thickness of positive photoresist film on the geometry of the PDMS replica from the reflowed microstructure. (B-G) typical bright field microscopic images of three generation cross-sections of channel for thickness of 55 and 85 $\mu m$, respectively. The reflow temperature was 130 °C and the reflow period was 70 sec. Scale bar is 100 $\mu m$ in (B-G).
Figure 6.7 The contact angle as a function of $H/W$. Crosses: $H = 55$ µm, Triangles: $H = 65$ µm, Squares: $H = 75$ µm, Circles: $H = 85$ µm. Black data points: $W = 200$ µm (1st generation), Green data points: $W = 158$ µm (2nd generation), Magenta data points: $W = 126$ µm (3rd generation). The blue solid curve is the prediction of the Eq. 6.2 with $c = 1$ and $f = 1$. The reflow temperature was 130 °C and the reflow period was 70 sec.

6.3.2 Variation of reflowed structure with reflow condition

With increasing heating temperature, the contact angle of the cross section for all generations decreased weakly (Fig. 6.8A). If the heating temperature was set at 130 °C, contact angle of the cross section for all generations decreased with increasing reflow period (Fig. 6.8B). The thickness of the positive photoresist was 65 µm before reflow. The trends are also shown on a plot of the contact angle with $H/W$ in Fig. 6.9A. With increasing heating energy (temperature and period of reflow), more solvent is lost from the photoresist, and the factor $f$ becomes smaller. As indicated in Fig. 6.2, the decrease in $f$ for a fixed $H/W$ leads to a decrease in the contact angle.
Figure 6.8 Variation of reflowed structure with varying reflow conditions. (A) Effect of heating temperature for 70 sec reflow on the contact angle of three generation branches of PDMS replica. (B) Effect of heating period during 130 °C reflow on the contact angle of three generation branches of PDMS replica.

Figure 6.9 Effect of temperature (A) and time (B) on the contact angle. The color scheme for the 1st, 2nd and 3rd generations are the same as Fig. 6.7. (A) Effect of temperature: Circles – 110 °C, Squares – 120°C, Triangles – 130°C; (B) Effect of reflow period: Circles – 50 sec, Squares – 70 sec, Triangles – 90 sec.

6.3.3 Variation of reflowed structure with SU-8 photoresist structure

Figures 6.6 and 6.7 show that the contact angle of 1st generation channel is lower than 80° for all conditions of film thickness. Our idea is to increase the contact angle by increasing $H/W$ via the insertion of an SU-8 layer beneath the AZ40XT film. SU-8 structures with various widths ($W_0 = 100 \, \mu m$ in Fig. 6.10A-C, and $W_0 = 150 \, \mu m$ in Fig. 6.10D-F) and height $H_0$ (20 \, \mu m in Fig. 6.10A, D; 35 \, \mu m in Fig. 6.10B, E; and 65 \, \mu m in Fig. 6.10C, F) were embedded below AZ40XT in order to increase the height of first generation of microchannel after reflow at 130 °C for 70 sec. The volume of SU-8 photoresist below the AZ40XT one increased with both the final width
and height, and the smoothest structures and contact angles were observed when width was \( W_0 = 100 \, \mu m \) and height was \( H_0 = 35 \, \mu m \). If the height was too small (e.g. \( H_0 = 20 \, \mu m \) and \( W_0 = 100 \, \mu m \)), the SU-8 layer did not affect the contact angle. When \( H_0 \) was too large (e.g. \( H_0 = 65 \, \mu m \) and \( W_0 = 100 \, \mu m \), or any of the explored heights for \( W_0 = 150 \, \mu m \)), the cross-sectional area of the negative photoresist was not completely covered by the positive photoresist, resulting in a sharp corner interfering with the semi-circularity of the cross-section. The best condition of SU-8 structure, i.e., \( W_0 = 100 \, \mu m \) and \( H_0 = 35 \, \mu m \), rendered the height of entire hybrid photoresist to be 75 \( \mu m \) (data not shown), resulting in \( H/W \) increasing from 0.325 (when \( H = 65 \, \mu m \) for AZ40XT photoresist without SU-8 insertion) to 0.375. The experimental findings thus agreed with the prediction that the semi-circular cross-section can be achieved when the initial \( H/W \) approaches 0.4 (Fig. 6.2).

Figure 6.10 Effect of varying width (100 \( \mu m \) in A - C, and 150 \( \mu m \) in D - F) and height (20 \( \mu m \) in A, D; 35 \( \mu m \) in B, E; and 65 \( \mu m \) in C, F) of first-layered SU-8 microstructure on the contact angle of the first generation of PDMS replica. The reflow temperature was 130 °C and the reflow period was 70 sec. Scale bar is 100 \( \mu m \) and applies to all images.

6.3.4 Effect of reflow on branching angle

To explore the ability to fabricate microchannels with circular cross-sections for multiple generations, various branching angles were designed and the cross-sections of resultant assembled PDMS channels were characterized. Following the optimized preparation condition (65 \( \mu m \)-thick positive photoresist before reflow), both second (Fig. 6.11C, D) and third (Fig. 6.11F, G, I, and J) generation of microchannel reflowed to the circular shape of their cross-sections. This happened for both branching angles, and thus, the branching angles that were
originally patterned in the photomask were maintained at 15° and 60° in the respective branching points in the assembled PDMS microchannel.

Figure 6.11 Reflowed structure of microchannel with various branching angles (15° and 60°) both from 1st to 2nd, and from 2nd to 3rd generation. The various angles were designed in AutoCAD software (A), and the resultant assembled PDMS channels were characterized at both 1st (B, C) and 2nd (D, E) branching points in side view (B, D) and cross-sectional view (C, E). The preparation condition of the microchannel followed the optimized recipe (65 μm-thick positive photoresist before reflow, reflow at 130 °C for 70 sec). Scale bar is 100 μm in (B-E).

6.3.5 Variation of dimensions of daughter channels of reflowed structure

To explore the ability to fabricate a microchannel with circular cross-sections for multiple generations, various dimensions of daughter channels were designed (160 and 140 μm for top and bottom second generation channels; 130, 110, 90 and 70 μm for 1st to 4th third generation channels from top to bottom in Fig. 6.12A), and branching angles were maintained as 45° for all branching points. A modified preparation recipe (65 μm-thick positive photoresist before reflow, reflow at 130 °C for 120 sec) was applied and cross-sections of resultant assembled PDMS channels were characterized (Fig. 6.12B-J). Both second (Fig. 6.12B-D) and most third (Fig. 6.12E-I) generation of microchannel reflowed to the circular shape of their cross-sections (aspect ratio within 0.9 and 1.1). Aspect ratio is defined as height over width of the cross-section. However, the smallest third generation channel
reflowed to an oval cross-section with aspect ratio of 1.14 (Fig. 6.12J), indicating the limitation of the reflow procedure. With the same reflow condition for all dimensions of the same level channels, the narrower channel reflowed to oval shape cross-section with higher aspect ratio. Therefore, further decrease of channel dimension would result in a cross-section shape away from circle.

Figure 6.12 Reflowed structure of microchannel with various dimensions of daughter channels both in second and third generation. (A) Top and bottom second generation channels were designed as 160 and 140 μm; 1st to 4th third generation channels from top to bottom were designed as 130, 110, 90 and 70 μm. The resultant assembled PDMS channels were characterized at both first (B-D) and the double second order branching points (top point in E-G; bottom point in H-J) in side view (B, E and H) and cross-sectional view (C, D; F, G; and I, J). The preparation condition of the microchannel followed the modified recipe (65 μm-thick positive photoresist before reflow, reflow at 130 °C for 120 sec). Scale bar is 100 μm in (B-J).

6.3.6 Effect of microgel progression on the redistribution of liquid

Full or partial occlusion of one or more pulmonary arteries leads to ventilation/perfusion (V/Q) matching abnormalities, which include extremely high or infinite V/Q values in the embolized region(s) but potentially decreased V/Q units in the non-occluded tissue, and hence impaired gas exchange (hypoxemia). The changes in V/Q after Acute Pulmonary Embolism (APE) are determined primarily by the redistribution of pulmonary blood flow, with a minor contribution from ventilation redistribution. Computational models have been used to simulate blood flow redistribution and V/Q mismatch during embolic pulmonary arterial occlusion[49]. Red blood cell transit times decreased below the minimum time for oxygen saturation (< 0.25 sec) and capillary pressures were observed to become high enough to initiate cell damage (which may result in edema) after ~ 80% of the
lung was occluded. In our studies, we used the branched microchannel as an in vitro model of pulmonary capillaries, by blocking branches of the microchannel with fibrin microgels (stiffness of 3.5 kPa). The effect of blockage of one or a few microfluidic channels in a network on the redistribution of liquid flow in the non-occluded regions is expected to shed light on mechanistic aspects of pulmonary embolism.

In a typical MF experiment, after the introduction of a fibrin microgel ($D_0 = 200 \mu$m) into the microchannel, the valve connected to the syringe containing the microgel suspension was closed, and the valve connected to upstream reservoir was opened. This caused the microgel to be driven into the MF channel under the imposed pressure drop and partially block the first branching point (Fig. 6.13A) at a pressure drop of 1500 Pa (Fig. 6.13B). Such pressure drop was not enough to entirely block the liquid flow within any branches of the microchannel, but the top two branches in the third generation received much more liquid (average velocity of 47 mm/min) compared to the bottom two branches (average velocity of 4 mm/min) (Fig. 6.13D). When pressure drop increased to 2100 Pa, the microgel progressed further into the bottom branch (Fig. 6.13C), and thus completely blocked the bottom two branches in the third generation (0 mm/min) while the top two branches experienced enhanced flow (304 mm/min). The redistribution of the liquid due to partial or complete blockage of branches mimics the variation of $V/Q$ in lungs, where embolized regions have an increased $V/Q$ ratio due to suppressed flow (bottom two branches at 1500 Pa) or even infinite value (called “dead space” in physiology) due to completely blocked flow (bottom two branches at 2100 Pa). On the other hand, regions proximal to the emboli receives more diverted flow (top two branches) which results in a decreased $V/Q$ ratio. In the extreme case, a pulmonary shunt may occur when the alveoli fill with fluid, causing parts of the lung to be unventilated although they are still perfused.
Figure 6.13 Effect of microgel progression on the liquid redistribution within the branched microchannels. A fibrin microgel of 200 µm (stiffness of 3.5 kPa) was introduced into the microchannel (A) and partially blocked the first branching point at 1500 Pa (B). The applied pressure further increased to 2100 Pa and the microchannel completed blocked the lower branch of the branching point (C). (D) Velocity of liquid flow within the 3rd generation channels (1st to 4th from top to bottom in (A)) were characterized by measuring the flow of 1µm FITC-polystyrene beads with high speed camera at 4000 frame per second. Blue dotted lines in B and C indicate the profile of the confined microgel within the channel. Scale bar is 100 µm in B and C.

6.3.7 Effect of blockage percentage of microgel on the redistribution of liquid

One or more fibrin microgels (stiffness of 3.5 kPa, size range between 190 and 220 µm) were introduced into the microchannel at the same pressure difference (2400 Pa), and the microgel(s) blocked (only) the third-generation channels in four different patterns as shown in Fig. 6.14. The average velocity of the liquid flow within open 3rd generation channel(s) was measured at 4000 fps under fluorescent mode according to various blockage patterns. Compared to the situation when only one of the four branches was blocked by a fibrin microgel (Fig. 6.14A), average liquid velocity increased with the percentage of blockage of total branches (Fig. 6.14B-D). As for two branches blockage situations, symmetric blockage (Fig. 6.14B) resulted in a higher liquid velocity than asymmetric blockage (Fig. 6.14C). Enhanced flow of unblocked channel(s) mimics the non-occluded regions in pulmonary network, the diverted flow leads to a decreased $V/Q$ ratio. We showed such enhancement was amplified with the percentage of blockage.
Fabricated branched structure was used mimic in vivo pulmonary arterial tree for the following several comparisons. According to data or estimates for each order of branching in the pulmonary tree in previous studies[50] from the cast of a human pulmonary arterial tree, diameter of the branches varies from 30 mm to ~ 10 μm with the decay in the generation of the branches. Therefore, the microchannel dimensions in our studies reside in the range above, and can be used to mimic a certain portion of the pulmonary arterial tree by matching the size and relative angle between branches to the anatomical results. In addition to the geometry, the applied pressure drop in our experiments is close to the pulmonary pressure which is within the range between 10 and 25 mmHg (1300 and 3300 Pa). Moreover, the detected liquid velocities in our experiments also fall into the range of average capillary blood speed (between 0.1 and 10 mm/s) based on experimental observations and theory of blood flow[51].

6.4 Conclusions

We have presented a modification of the reflow technique for fabricating circular channels that allows the generation of channels of different diameters from a single master. Our method relies on the insertion of a layer
of a second, non-melting photoresist (SU-8) under the reflow photoresist layer only at selected places in the master where a larger diameter is desired. This increases the initial height to width ratio of the reflow resist in those regions relative to other regions where the non-melting photoresist is absent. Previous attempts in the literature have produced branched networks, but the diameters of the parent and daughter channels have either been constant, or the variation in channel diameters in the network has been limited. In this work, we have varied channel diameters over a wider range from 70 to 200 μm, while maintaining nearly circular cross-sectional shapes. We demonstrated the utility of such a network as a model for the pulmonary artery system by trapping a fibrin clot in one branch of the network, and measuring the redistribution of the flow in other branches of the network at various total pressure drops imposed across the network.
References


Chapter 7
Summary and outlook

7 Summary and outlook

7.1 Summary

This thesis makes a first step towards the delineation of the relationship between composition, dimensions, and mechanical properties of occlusive blood clots, and their resistance to thrombolysis. To establish these connections, a “double-modeling” system was developed to investigate the motion of a surrogate embolus (a spherical microgel) through an artificial blood vessel (a microchannel with a narrow constriction). The dimensions of both models and the experimental conditions were comparable to those in vivo.

Our initial effort focused on the entrance and passage of an individual agarose microgel through a narrow tube (microchannel). By varying the applied pressure drop, we changed microgel position in the MF channel, as well as the loss of water by the microgels. These changes agreed with predictions made on the basis of the theory of linear elasticity. The results of this work were extended to a comprehensive study of the behavior of microgels with various dimensions and mechanical properties in narrow microchannels with varying geometries. Based on experimental findings, we established universal principles governing the flow of microgels under spatial constraints. We also showed a non-monotonic change in the flow rate of liquid through the occlusive microgel, which was governed by its progressive confinement in the tapered region of the channel. The observed generalized relationships developed as a result of this work were in excellent agreement with a theoretical model developed for biaxial deformation of the microgel. Our results were universal for unentangled gels formed by flexible polymer chains, swollen in a good solvent and cross-linked permanently on the time scale of experiments. As long as these criteria are met, the behavior of the gel does not depend on the chemical composition of the polymer or the solvent. In terms of thromboembolism and thrombolysis, our results may help in gaining a qualitative understanding of the physical principles governing these phenomena, especially, for convection-enhanced delivery of liquid to and through an occlusive thrombus.

The viscoelastic behavior of occlusive gels was investigated based on the relaxation of agarose microgels that were released from the constriction to a channel-at-large to gain their unbound state. A nontrivial
phenomenon was observed: the microgels recovered their spherical shape, first, and subsequently, swelled to attain the initial volume. The mechanism of such two-step recovery is yet to be discovered. It was also found that when sufficient time was not provided for the complete size and shape relaxation, a partly deformed microgel could be forced into the constriction under a lower applied pressure difference.

To further mimic and study thromboembolism and thrombolysis, fibrin microgels with different morphologies and mechanical properties were prepared by varying fibrinogen-to-thrombin ratio in the precursor droplets. These microgels were inserted into a narrow biomimetic microchannel with a circular cross-section. Occlusive microgels formed by thicker fibers with fewer branching points exhibited weaker elasticity and a larger degree of irreversible deformation in comparison with microgels formed by a denser network of thinner fibers, both subjected to the same strain. In addition, the gels with former structure are prone to liquid permeation and fibrinolysis. Although more complicated relationships between the structure, viscoelasticity, and fibrinolytic resistance of blood clots exist in vivo, our study proposes a very promising platform for fundamental in vitro studies of the implications of this relationship on treatment of thromboembolism.

Lastly, bifurcating microchannels with nearly circular cross-sections throughout multiple channel generations were successfully fabricated using a cost-efficient soft lithography method, thus providing a model for in vivo microvascular network. When a portion of the branching channels was blocked with fibrin microgel, we observed redistribution of the liquid within the microchannel network. Such flow rearrangement is closely mimicking the phenomena occurring in pulmonary embolism, with abnormality of blood perfusion.

7.2 Outlook

Our current MF platform is a constant pressure system, which better mimics the pressure-driven flow of blood across the vasculature in comparison with a constant flow system. Arterial flow, however, is pulsatile with blood flow ranging from 0, to more than twice the average blood velocity during each circulative cycle[1]. Although the effect of mechanical stress of streaming fluids (originating from the physiological flow) on clot lysis has been considered frequently[2,3], few investigations have been conducted on
thrombolysis with a pulsatile flow system. We plan to generate pulsatile flow velocities by a computer-controlled pump and study fibrinolysis, as well as the embolic effect of the fibrin clots. Such studies help to understand the interaction of thromboembolic disease and hemodynamic parameters, which might lead to far-going implications for clinical treatment.

The predictive power of the universal principles as described in Chapter 4 is yet to be tested on other classes of hydrogels, including those formed by (i) semi-flexible or rigid polymer chains, (ii) entangled chains, (iii) temporary cross-linkers, and (iv) gels swollen with theta-solvents or – in case of semi-flexible chains – marginal solvents. Importantly, such tests on additional types of hydrogels can also serve as a technique to determine their Young’s moduli, complementing other techniques, e.g., atomic force microscopy (AFM), micropipette aspiration.

Besides the occlusive behavior, the relaxation behavior of microgels is also correlated with the viscoelasticity of the material, and is especially important for biomaterials in vivo, which are exposed to different external forces including pulsatile hydrodynamic as mentioned above. The nontrivial phenomenon of agarose microgel relaxation deserves further investigation, both experimentally and theoretically. For small deformations, shape relaxation is correlated only with the shear modulus of the material, while volume relaxation is governed by both bulk and shear modulus. What we are observing is that non-spherical components of deformation vanish faster than the spherically symmetric components of deformation. Mathematically, this means that the eigenvalues of relaxation of non-spherical modes are larger than those for the spherical modes. This needs to be investigated in more detail. In terms of time scales for fibrin microgel relaxation as reported in Chapter 5, we noted that a second order exponential decay (i.e., including $\tau_1$ and $\tau_2$) fitted the experimental data much better than single order one (i.e., with only $\tau'_1$) (data not shown), and $\tau_1$ is an order of magnitude higher than $\tau_2$. Such data fitting indicates that more than one type of relaxation is involved in the recovery of microgel properties (shape, volume, and birefringence). Moreover, $\tau_1$ for MMs are the longest, in comparison with those of SMs and RMIs, which is not intuitive and needs deeper understanding.

Another relaxation-induced phenomenon observed in fibrin microgels also deserves investigation. As we found for agarose microgel in Chapter 2, incomplete recovery of the microgel shape and size leads to easier passage of the microgel through a second constriction of the same size at the same pressure. Such behavior
of fibrin microgels in our MF channels would be a simulation of in vivo emboli that encounter subsequent branching points within microvascular network. An irreversible deformation before the entrance of the emboli into downstream capillaries would render the emboli less occlusive than they would be had they not negotiated a narrow vessel. This phenomenon can be easily studied on channels prepared with the fabrication technique discussed in Chapter 6.

To enhance the biomimetic capability of the current “double-modeling” platform, a closer mimicry of both blood clots and blood vessels is required. The mechanical properties of artificial clots with various compositions needs to be further tested on the platform. For collagen gel, a typical fibrillar biopolymer gel, models describing the relationship between the elastic properties and fibrillar structures (averaged fiber diameters\(^4\), crosslink density\(^5\)) have been established. Such mathematical correlation for fibrin gels are yet to be confirmed. A closer analogy with clots in vivo will be achieved by incorporating erythrocytes into fibrin gels, and a significant increase in the moduli and the Poisson ratio is expected\(^6\), potentially leading to a more difficult translocation through constriction, smaller volume changes and a faster recovery after confinement. The passage and deformation of clots through stenosis will also be strongly affected by the deformability of the MF channel. Channels made of Ultra-soft PDMS or hydrogels will enlarge upon emboli occlusion, which may result in lower critical pressure drop, and less significant volume reduction of the emboli upon confinement. A modification that will take this platform a step closer to blood vessels is the coating of the inner surface of MF channel with endothelial cells (ECs), in order to study the interaction between fibrin and ECs. In pulmonary embolisms, for example, embolic incorporation mechanically impinges the EC surface, and the resulting vascular remodeling is known to regulate a series of phenotypic properties\(^7\).

A key advantage of our biomimetic platform originates from the detailed studies of artificial soft clots subjected to two-dimensional compressional strain, which is similar to in vivo thromboembolism. The translocation of the microgel from the channel-at-large to the constriction through a tapered region leads to a continuously increasing radial strain of the clot. In agarose microgels, the imposed radial compressional strain resulted in a significant axial elongational strain. But fibrin microgels responded with a weak axial elongation even for large radial compressional strain, deforming almost like a sponge. Although we have observed the re-orientation of fibrillar structure of the fibrin microgel under polarized microscopy, a real-time demonstration of strain-hardening associated with axial elongation is required to validate this transition.
As described in Chapter 1, unfolding of fibrin molecules is directly associated with stiffening of fibrin clots, and can be observed in situ both qualitatively and quantitatively. The secondary structure change, that is, α-helix to β-sheet transition, has been quantified by other groups by Fourier Transform infrared spectroscopy[8]. In the future, we will integrate this characterization technique with our current platform, and simultaneously check for the existence of strain-hardening of fibrin microgels.

In addition to mechanical properties, the fibrinolytic resistance of the clots also depends on the strain, in agreement with previous reports on uniaxial elongated fibrin clot and our results described in Chapter 5. Owing to the progressive confinement in the tapered region, the strain is not homogeneously distributed on the entire fibrin microgel, and thus the effect of strain on lysis rate is yet to be further discovered. We note that the non-uniform distribution of strain has biological relevance, as it exists in occlusive clots in vivo. A detailed study would aim at studies of two phases of lysis, namely, plasminogen activation and clot digestion. We expect that the first stage would be governed by exposure of hydrophobic groups of fibrin to convection-driven plasminogen activator. Therefore, the examination of α-helix to β-sheet transition would provide substantial evidence of strain-hindered plasminogen activation. Measurements of plasmin diffusivity in both radial and longitudinal direction through the occlusive fibrin clot by fluorescence recovery after photobleaching (FRAP) would enable us to examine the effect of strain on the second lytic stage.

A detailed investigation of dynamic process of thrombolysis needs to be implemented, based on the current experimental findings as shown in Chapter 5. In our current data, the t-PA fluorescence signal can only be detected when a significant amount of t-PA has accumulated throughout the fibrin microgel (~ 30 min after lysis starts). A relatively weak fluorescent staining of t-PA molecules restricts monitoring the behavior of the plasminogen activator during the initial stage of lysis, i.e., t-PA aggregation on the fibrin surface. In addition, a lack of fluorescent labeling of fibrin impedes a clear definition of the boundary between remaining fibrin and biodegradable products during the progressive digestion. The deficiency of fibrin labeling also limits the observation of preferential lysis direction (i.e., the lysis rate is faster in radial or longitudinal direction), if any, due to heterogeneous deformation of the microgel in our MF channel. To this end, an improved fluorescent intensity of t-PA and a fluorescent labeling of fibrin will be applied to our system. Importantly, the recanalization of liquid during lysis needs further investigated by simultaneously monitoring the liquid flow rate through the confined microgel, integrated with the FRAP
method we established in Chapter 4. The intercorrelation between liquid restoration, lysis period, volume and fibrillar structure of the remaining clot, as well as the adopted thrombolytic agent, will shed light on the reperfusion therapy of thromboembolism. A comprehensive mathematical model will be developed building on the work of Diamond and co-workers\cite{2,9} to incorporate the effect of radial compression on permeability and lytic ability of fibrin fibers.

The next step in developing a model of the microvascular system is to simulate collateral circulation around an obstructive microfluidic channel. The vascularization of collateral vessels, \textit{in vivo}, is induced by pathological conditions, e.g., atherosclerosis, and the newly developed vessels minimizes the impact of thrombotic occlusion by carrying the blood flow. We aim to fabricate mesh-like capillary bed which would provide flow pathways during embolism before the recanalization of the main vessel. The network-like microchannels obtained by the reflow method (described in Chapter 6) can be used to study embolic and thrombolytic effect, which would significantly strengthen the biological relevance of our work.

Owing to the consistency and high fidelity of cylindrical MF channels prepared as described in Chapter 6, commercialization of the MF platform is possible. To note, the replicated material is not limited to PDMS, and can be extended to other polymers and hydrogels, as long as an appropriate alignment and sealing can be performed between two identical replica layers. Such a tubular network serves as a cost-efficient, highly reproducible \textit{in vitro} model in biological and biomedical applications. One of the potential fields would be embolization therapies which utilize deliberate obstruction of blood vessels with gel beads and are used for treating hemorrhage or specific types of cancer\cite{10}. Calibrated microspheres are provided in accordance with the diameter of the targeted blood vessel in order to completely block the vessel. The controlled geometry parameters of the MF channels promise the feasibility of such test. Our MF model is also a convenient platform for the screening of drugs for thrombolytic therapy. The proposed MF system is not limited to the combination of plasminogen activator and thrombi (i.e., t-PA and pure fibrin gels, respectively) investigated in this work. A variety of thrombolytic agents, e.g., streptokinase, urokinase, anistreplase, alteplase, reteplase and mixtures thereof can be administrated on occlusive thrombi made from plasma. Variation of plasma sources would determine the composition of occlusive clots (in the presence of platelets, leukocytes, and erythrocytes). Therefore, comprehensive \textit{in vitro} studies of drug efficacy and efficiency on certain types of thrombi will provide valuable information to thromboembolic therapy.
References


Appendices

Appendix to Chapter 3

In this appendix, we modify a previous approach\(^1\) to determine the pressure difference across the microgel and the volume change experienced by the microgel corresponding to a static steady state in the tapered section of the constriction.

1.1 Physical Model

Consider a microgel trapped within a microchannel in static force equilibrium as depicted in the Fig. 3.9 in Chapter 3. In order to support the microgel in this position, the pressure difference across the microgel must be balanced by a pressure \(P_w\) from the wall in the contact zone \(S_c\). Employing a pressure balance over the microgel surface, we have

\[ \mathbf{i} \cdot \int_{S_c} P(-\mathbf{n}) dS = \int_{S_L} P_L(-\mathbf{i} \cdot \mathbf{n}) dS + \int_{S_c} P_w(-\mathbf{i} \cdot \mathbf{n}) dS + \int_{S_R} P_R(-\mathbf{i} \cdot \mathbf{n}) dS = 0. \]  

(A1.1)

Here, \(\mathbf{i}\) is the unit vector in the axial (\(x\)) direction. \(P_L\) and \(P_R\) are the pressures acting on the left and right spherical caps \(S_L\) and \(S_R\) respectively. Since \(P_L\), \(P_R\) and \(P_w\) are constant pressures acting on their respective surfaces, we can write

\[ P_L \int_{S_L} \mathbf{i} \cdot \mathbf{n} dS + P_w \int_{S_c} \mathbf{i} \cdot \mathbf{n} dS + P_R \int_{S_R} \mathbf{i} \cdot \mathbf{n} dS = 0. \]  

(A1.2)

It is straightforward to show that \(\int_{S_L} \mathbf{i} \cdot \mathbf{n} dS = \pi D_L^2 / 4\), \(\int_{S_c} \mathbf{i} \cdot \mathbf{n} dS = -\pi D_R^2 / 4\) while \(\int_{S_R} \mathbf{i} \cdot \mathbf{n} dS = \pi \left(D_L^2 - D_R^2\right) / 4\). Thus, we derive the following expression for \(P_w\)

\[ P_w = \frac{P_L D_L^2 - P_R D_R^2}{D_L^2 - D_R^2}. \]  

(A1.3)

We can now employ a linear elastic model with the constitutive equation\(^2\),

\[ \sigma_{ij} = K \delta_{ij} \varepsilon_{kk} + 2G \left( \varepsilon_{ij} - \frac{1}{3} \delta_{ij} \varepsilon_{kk} \right), \]  

(A1.4)
to relate these externally applied stresses to the internal elastic stresses. Here, $\sigma_{ij}$ is the Cauchy stress, $\varepsilon_{ij}$ is the strain tensor, $G$ is the shear modulus of the microgel, and $K$ is the bulk modulus. Following Eq. 4 and 5 in Ref. 49\[1\], the stress balance may be implemented in the $x$ and $r$ directions to yield the following equations:

$$\Delta P_{stat} = K \left( 2\varepsilon_{rr} + \varepsilon_{xx} \right) - \frac{4}{3} G \left( \varepsilon_{rr} - \varepsilon_{xx} \right)$$  \hspace{1cm} (A1.5)

$$P_w = \frac{(p_L + p_R)}{2} = K \left( 2\varepsilon_{rr} + \varepsilon_{xx} \right) + \frac{2}{3} G \left( \varepsilon_{rr} - \varepsilon_{xx} \right)$$  \hspace{1cm} (A1.6)

Here, $\Delta P_{stat} = p_L - p_R$, and $\varepsilon_{rr}$ and $\varepsilon_{xx}$ are the normal strains in the radial and axial directions. We approximate the radial strain experienced by the microgel as

$$\varepsilon_{rr} = \frac{1}{L} \int_0^L \frac{r}{D_0} dx$$  \hspace{1cm} (A1.7)

where $r$ is the variation of the radius of the microgel with the axial position $x$ from $x = 0$ to $x = L$. It may be verified that when the microgel is a sphere of original diameter $D_0$ (i.e. in its undeformed configuration), the radial strain is identically zero. Note that the balances in equations (A1.5) and (A1.6) have been written for shallow taper angles, which is a reasonable for the $15^\circ$ taper considered in this chapter.

The two unknowns in equations (A1.5) and (A1.6) are $\varepsilon_{xx}$ and $\Delta P_{stat}$, and we can solve these simultaneous equations to yield the unknowns as

$$\Delta P_{stat} = \frac{36GK (D_L^2 - D_R^2)}{(4G + 3K)(D_L^2 + D_R^2) + 2(2G - 3K)(D_L^2 - D_R^2)} \varepsilon_{rr},$$  \hspace{1cm} (A1.8)

and

$$\varepsilon_{xx} = 2 \varepsilon_{rr} \frac{(2G - 3K)(D_L^2 + D_R^2) + 2(G + 3K)(D_L^2 - D_R^2)}{(4G + 3K)(D_L^2 + D_R^2) + 2(2G - 3K)(D_L^2 - D_R^2)}. \hspace{1cm} (A1.9)$$

The volume change $\Delta V$ experienced by the particle is related to the strains $\varepsilon_{rr}$ and $\varepsilon_{xx}$ by the equation

$$\frac{\Delta V}{V_0} = 2\varepsilon_{rr} + \varepsilon_{xx} = \frac{24D_L^2 D_R^2 \varepsilon_{rr}}{\left( 4 + \frac{3K}{G} \right)(D_L^2 + D_R^2) + 2 \left( 2 - \frac{3K}{G} \right)(D_L^2 - D_R^2)}. \hspace{1cm} (A1.10)$$
Here $V_0$ is the initial volume of the gel, defined as

$$V_0 = \frac{\pi D_0^3}{6} \quad (A1.11)$$

The relative volume of the microgel, $V / V_0$, is

$$\frac{V}{V_0} = 1 + \frac{24D_0^2\epsilon}{(4 + \frac{3K}{G})(D_L^2 + D_H^2) + 2 \left( 2 - \frac{3K}{G} \right)(D_L^2 - D_H^2)} \quad (A1.12)$$

Before we can determine the position of the microgel and the volume change as a function of $\Delta P_{stat}$, we need to delineate two geometries: the geometry of the microchannel, and the geometry of the microgel.

### 1.2 Microchannel geometry

The channel constriction spans from $x = -L_c$ to $x = L_c$. The diameter of the channel, $d_{chan}$, varies smoothly from $d$ the value of in the channel-at-large to $d_c$ within the constriction, and then again back to the diameter $d$ of the channel-at-large on the right (see Fig. 3.9). This can be approximated by the equation

$$d_{chan}(x) = d + \frac{d - d_c}{2} \left\{ \tanh \left[ \frac{(x - L_c - \frac{W}{2})}{W/2} \right] - \tanh \left[ \frac{(x + L_c + \frac{W}{2})}{W/2} \right] \right\} \quad (A1.13)$$

Here, $W$ is the length of tapered section

$$W = \frac{d - d_c}{2\tan\alpha} \quad (A1.14)$$

where $\alpha$ is the entrance angle to the constriction. Note that although we have approximated the entire geometry shown in Fig. 3.9, only the left half is actually employed in the simulations.

### 1.3 Microgel geometry

To calculate the volume of the microgel, we break down the shape into three parts (see Fig. 3.9): (a) spherical cap on the upstream end, $S_L$, whose volume is $V_L = \frac{\pi h_L}{6} \left( \frac{3D_L^2}{4} + h_L^2 \right)$; (b) a central contact region or waist region, $S_C$, overlapping with the constriction geometry, whose volume is $V_W =$
\[ \int_{X_L}^{X_R} \frac{\pi d_{\text{chan}}^2}{4} \, dx; \text{ and (c) a spherical cap on the downstream side, } S_R, \text{ with a volume } V_R = \frac{\pi h_R}{6} \left( \frac{3D_R^2}{4} + h_R^2 \right). \]

Thus the volume of the microgel is

\[ V = \frac{\pi h_L}{6} \left( \frac{3D_L^2}{4} + h_L^2 \right) + \int_{X_L}^{X_R} \frac{\pi d_{\text{chan}}^2}{4} \, dx + \frac{\pi h_R}{6} \left( \frac{3D_R^2}{4} + h_R^2 \right) \quad (A1.15) \]

The cap heights \( h_L \) and \( h_R \) are determined by assuming the microgel shape to merge smoothly from the spherical cap region into the waist. This requires that the tangents to the spherical cap and the waist at the point of contact be identical. Thus,

\[ h_L = \frac{d_L}{2} \left[ -\frac{d_{\text{chan}}}{dx} \bigg|_{X=X_L} + \frac{1}{\left( \frac{d_{\text{chan}}}{dx} \bigg|_{X=X_L} \right)^2} \right] \quad (A1.16) \]

and

\[ h_R = \frac{d_R}{2} \left[ -\frac{d_{\text{chan}}}{dx} \bigg|_{X=X_R} + \frac{1}{\left( \frac{d_{\text{chan}}}{dx} \bigg|_{X=X_R} \right)^2} \right] \quad (A1.17) \]

### 1.4 Numerical procedure

The procedure for determining the relationship between the position of the microgel and \( V/V_0 \) with \( \Delta P_{\text{stat}} \) is as follows.

1) Guess the bulk and shear moduli, \( K \) and \( G \).

2) For a given value of \( X_L \), calculate \( D_L = d_{\text{chan}}(X_L) \) and \( h_R \) from Eqs. (A1.13) and (A1.16) respectively.

3) Guess the value of \( X_R \) (greater than \( X_L \), obviously).

4) Calculate \( D_R = d_{\text{chan}}(X_R) \) and \( h_R \) from Eqs. (A1.13) and (A1.17) respectively.

5) Calculate the volume of the microgel using Eq. (A1.15), and hence its relative volume \( V/V_0 \).

6) Calculate the relative volume from Eq. (A1.12).

7) Compare the relative volumes from steps 5 and 6. If the difference is below a tolerance, say \( 10^{-5} \), go to step 8; if not, go to step 3 to re-guess the value of \( X_R \), and repeat steps 4 through 7.

8) Compute \( \Delta P_{\text{stat}} \) from Eq. (A1.18) and \( V/V_0 \) from Eq. (A1.15).
9) Compute the locations of the back and front edges of the microgel as \( X_b = X_L - h_L \) and \( X_f = X_e + h_e \), respectively. Therefore, compute the approximate center of the microgel as \( X_c = \left( X_b + X_f \right) / 2 \).

10) Repeat steps 2 through 7 for different values of \( X_L \), thus generating an array of values for \( X_c \), \( \Delta P_{stat} \) and \( V/V_0 \).

11) Compute the deviation of the variation of \( X_c \) and \( V/V_0 \) with \( \Delta P_{stat} \) obtained in step 8 with the experimental trends in Fig. 3.4B and Fig. 3.8C.

12) Adjust the values of \( K \) and \( G \), and repeat steps 2 to 11, so that the deviation between experiment and theory is minimized.

Note that the bulk and shear moduli are related to the Young’s modulus and Poisson ratio by the equations

\[
K = \frac{E}{3(1-2\nu)}, \quad G = \frac{E}{2(1+\nu)}.
\]  

(A1.18)

Appendix to Chapter 4

2.1 Determination of the flow rate of water through the microgel trapped at the entrance to the constriction

To determine the average velocity, \( U \), of the fluid flowing through the microgel from the CLSM data, the normalized first axial moment \( M_1/M_0 \) of the cross-section-averaged fluorophore concentration, defined as

\[
\frac{M_1}{M_0} = \frac{\int_0^l \int_0^d \frac{d}{2} x [I(x,r) - I_0(x,r)] r dr dx}{\int_0^l \int_0^d [I(x,r) - I_0(x,r)] r dr dx},
\]  

(A2.1)

was calculated from the CLSM images taken at time intervals of 5 s. In equation (A2.1), \( I(x,r) \) is the fluorescence intensity distribution; \( x \) and \( r \) are the axial and radial positions coordinates; and \( l \) and \( d \) are the length and diameter of the microchannel in the image (see Fig. 4.3A). The double integrals in equation (A2.1) were calculated in MATLAB using the trapezoidal rule[3]. As shown in the next section, when the fluorescence intensity profile after bleaching is nearly a square pulse (that is, the 2D projection of the
bleached region is nearly a rectangle), and the fluorophore distribution is axisymmetric, $M_1/M_0$ can be approximated by the following function of time

$$M_1/M_0 = X_0 + U t. \hspace{1cm} (A2.2)$$

The derivation of the equation (A2.2) is given below. In equation (A2.2), $X_0$ is the intercept of $M_1/M_0$ at $t = 0$. Since the radial dependence of fluorescence intensity can be measured either from the top half, or the bottom half of the CLSM image (the distribution is symmetric), the average value of $M_1/M_0$ (shown with black squares in Fig. 4.3B) from the two halves was fit to equation (A2.2); this is shown in Fig. 4.3B. The average velocity, $U$, of the liquid flowing through the trapped microgel was obtained as the slope of the plot of $M_1/M_0$ vs. $t$, and found to be $0.35 \pm 0.001 \text{ μm/s}$ in the experiment shown in Fig. 4.3. The flow rate, $Q$, of the liquid was determined as $Q = \pi (d/2)^2 U = 4019 \pm 14 \text{ μm}^3/\text{s}$, where $d/2$ is the radius of the microchannel, measured from the CLSM images to be $60 \text{ μm}$.

The above procedure was implemented for a range of pressure differences varying from the value required to block the microchannel, up to $\Delta P_{\text{max}}$. For each pressure difference, the FRAP experiment was repeated three times. Between repetitive FRAP measurements, a 5 min time interval was allowed for the system to reach a steady state. The variation in the average flow rate of the liquid through the microgel as a function of pressure difference is shown in Fig. 4.6. For pressure difference below 2000 Pa, the flow rate of water was high, due to its leakage through the gaps between microchannel walls and the microgel. The estimation of this effect is given in Appendix 2.2.

### 2.2 Interpretation of photobleaching data

In the derivation of equation (A2.2), we followed the work of Aris\[4\]. Consider the pressure-driven flow of a Newtonian fluid through a circular tube. The flow occurs in the $x$ direction, and $r$ and $\theta$ are the cross-sectional coordinates in the polar form. The governing equation for any disturbance, $\Delta \rho$, in the unbleached fluorophore concentration distribution is the time-dependent convective diffusion equation

$$\frac{\partial \Delta \rho}{\partial t} + u \frac{\partial \Delta \rho}{\partial x} = D_\ell \nabla^2 \Delta \rho + D_t \frac{\partial^2 \Delta \rho}{\partial x^2}. \hspace{1cm} (A2.3)$$
Here, $D_t$ is the diffusion coefficient of the fluorophore, $x$ is the direction of flow, and $u$ is the velocity in the flow direction

$$u = U \left(1 - \frac{r^2}{(d/2)^2}\right), \quad (A2.4)$$

where $U$ is the average velocity through the tube. The operator $\nabla_2$ in equation (A2.3) is the gradient in the cross-sectional coordinates $r$ and $\theta$. Note that $\Delta \rho$ is a disturbance in the concentration of the fluorophore. For example, if the initial concentration of the fluorophore is $\rho_0$ and the concentration at a later time is $\rho$, then the disturbance, $\Delta \rho$, in the concentration is defined as $\Delta \rho = \rho - \rho_0$. In the experiment, the disturbance is introduced in the form of a rectangular region by photobleaching, and is, therefore, non-zero only in that region. As shown in the derivation that begins in the next paragraph, the axial velocity of the center of mass of the concentration disturbance is equal to the average velocity of the fluid. This result is straightforward to understand, when diffusion is absent, but is valid even when there is diffusion-induced radial and axial smearing of the concentration, which can be explained as follows. Since the initial fluorescence intensity distribution produced by photobleaching is rectangular, it is symmetric about the center of mass at $t = 0$. As time proceeds, convection advances to the two edges of the rectangle to the right via the parabolic velocity profile, thereby establishing concentration gradients in the radial direction (see Fig. 4.6A). At the left edge, there is radial diffusion of the fluorophore from the center towards the walls, along with axial diffusion in the flow direction. Conversely, at the right edge, the fluorophore diffuses radially from the walls to the center, and axially - in the direction opposite to the flow. The key is that the diffusional distortions produced at the two edges complement each other exactly, their axial moments sum to zero, and do not contribute to the overall axial motion of the center of mass of the distribution. This is demonstrated more rigorously in the derivation below.

Multiplying equation (A2.3) by $x^n$, and integrating in $x$ from 0 to $l$ yields

$$\frac{\partial C_n}{\partial t} = nuC_{n-1} + D_t \left[\nabla_2^2 C_n + n(n-1)C_{n-2}\right], \quad (A2.5)$$

Here, $C_n$ is the $n^{th}$ axial moment of $\Delta \rho$, defined as

$$C_n = \int_0^l \Delta \rho x^n \, dx. \quad (A2.6)$$
For example, \( C_0 \), the zeroth axial moment of \( \Delta \rho \), is

\[
C_0 = \int_0^l \Delta \rho \, dx. \tag{A2.7}
\]

In deriving equation (A2.5), we have assumed that the concentration disturbance at the two ends of the tube, \( x = 0 \) and \( x = l \), is negligible. This is a valid assumption provided that the photobleached region far from the edges of the tube throughout the duration of the experiment. This condition was maintained in FRAP experiments. By integrating equation (A2.5) over the cross-section of the conduit, we obtain

\[
\frac{dM_n}{dt} = n \int_0^l u C_{n-1} \, dy \, dz + D_t n (n-1) M_{n-2}, \tag{A2.8}
\]

where \( M_n \) is the cross-section-integrated value of \( C_n \),

\[
M_n = \int_0^l C_n \, dA, \tag{A2.9}
\]

where \( dA \) is an elemental cross-sectional area.

For \( n = 0 \), equation (A2.8) becomes

\[
\frac{dM_0}{dt} = 0,
\]

implying that

\[
M_0 = \int_0^l C_0 \, dA = \text{constant, independent of time.} \tag{A2.10}
\]

Examination of equation (A2.5) for \( n = 0 \) gives the governing equation for \( C_0 \) (equation (A2.7))

\[
\frac{\partial C_0}{\partial t} = D_1 \nabla_2^2 C_0, \tag{A2.11}
\]

with the boundary condition

\[
\mathbf{n} \cdot \nabla_2 C_0 \bigg|_S = 0, \tag{A2.12}
\]

the integral constraint from equation (A2.10),
\[ \iint_S C_0 dA = M_0, \quad (A2.13) \]

and the initial condition

\[ C_0(r, \theta, 0) = \int_0^1 \Delta p(x, r, \theta, 0) dx = \Gamma(r, \theta). \quad (A2.14) \]

The function \( \Gamma(r, \theta) \) is obtained by axially integrating the initial concentration distribution \( \Delta p(x, r, \theta, 0) \).

The above problem has the following eigenfunction expansion solution

\[ C_0(r, \theta, t) = \frac{M_0}{A} + \frac{M_0}{A} \sum_{j=0}^{\infty} \left[ B_j \exp \left( -\mu_j \frac{D_\tau}{(d/2)^2} \right) \phi_j(r, \theta) \right]. \quad (A2.15) \]

The functions \( \phi_j \) and \( \mu_j \) are the eigenfunctions and eigenvalues, respectively, of the following linear problem

\[ \nabla^2 \phi_j = -\mu_i \phi_j, \quad (A2.16) \]

with the boundary condition

\[ n \cdot \nabla \phi \bigg|_S = 0. \quad (A2.17) \]

The constants \( B_j \) are derived from the initial condition for \( C_0 \) in equation (A2.14) using biorthogonality condition,

\[ \iint_S \phi_j \phi_i dA = \begin{cases} 0 & \text{for } i \neq j, \\ \iint_S \phi_j^2 dA & \text{for } i = j. \end{cases} \quad (A2.18) \]

as

\[ B_j = \begin{cases} \frac{\frac{A}{M_0} \iint_S \Gamma \phi_j dA}{\frac{\iint_S \phi_j^2 dA}{M_0}} & , \text{for } j \neq 0 \\ -1 + \frac{1}{M_0} \iint_S \Gamma dA & , \text{for } j = 0. \end{cases} \quad (A2.19) \]

Note that \( \Gamma \) is the axial integral of the initial concentration distribution (see equation (A2.14)). Since \( \iint_S \Gamma dA = M_0 \), the constant \( B_0 = 0 \). In addition, for the special case of a square pulse in the initial concentration profile that is imposed in the experiment, \( B_j = 0 \) for all \( j \neq 0 \), as well, implying that \( B_j = 0 \).
for all $j$. Note that for a square pulse, $\Gamma$ is independent of the cross-sectional coordinates. The null eigenfunction of the operator in equation (A2.16) (i.e. $\mu_{\phi} = 0$) is $\phi_{\phi} = 1$, and satisfies the following biorthogonality condition
\[
\iint_S \phi_{\phi} \phi_{j} dA = \iint_S \phi_{j} dA = 0 \quad \text{for} \quad j \neq 0.
\] (A2.20)

Equation (A2.20), combined with equation (A2.19), gives $B_{j} = 0$ for $j \neq 0$.

Since $B_{j} = 0$ for $j$, the solution for $C_{0}$ reduces from equation (A2.15) to:
\[
C_{0}(r, \theta, t) = \frac{M_{0}}{A}.
\] (A2.21)

Expressing equation (A2.8) for $n = 1$ yields
\[
\frac{dM_{1}}{dt} = \iint_{S} uC_{0}dA.
\] (A2.22)

Substituting for $C_{0}$ from equation (A2.21) gives
\[
\frac{dM_{1}}{dt} = \iint_{S} u \left( \frac{M_{0}}{A} \right) dA = M_{0} \left( \frac{1}{A} \iint_{S} udA \right) = M_{0} U.
\] (A2.23)

Dividing the above equation by $M_{0}$, we obtain
\[
\frac{d \left( M_{1} / M_{0} \right)}{dt} = U.
\] (A2.24)

Integration w.r.t. time results in the equation
\[
M_{1} / M_{0} = X_{0} + Ut.
\] (A2.25)

Thus, by fitting the temporal variation in the ratio $M_{1}/M_{0}$ to the linear equation (A2.15), we obtain $U$ as the slope. For a square pulse at $t = 0$, the spatial distribution of the fluorophore concentration is axisymmetric at every instant, which enables the description of the cross-sectional variations of the fluorophore concentration with only the radial coordinate, $r$. The elemental area, $dA$, for the double integral is $2\pi r dr$.

With this simplification, the definition of $M_{1}/M_{0}$ given in equation (A2.1) can be used.
In practice, the assumptions of axisymmetry and square pulse are not exact. To be more accurate, all the eigenmodes in the solution of $C_0$ in equation (A2.15) (except the $j = 0$ mode) have to be considered, which would result in the following expression for $M_1/M_0$:

$$\frac{M_1}{M_0} = X_0 + Ut + \frac{U (d/2)^2}{D_t} \sum_{j=1}^{\infty} b_j \left[ 1 - \exp \left( -\mu_j \frac{D_t}{(d/2)^2} \right) \right].$$ \hspace{1cm} (A2.26)

A linear regression of equation (A2.26) will provide the value of $U$. However, the calculation of $M_1/M_0$ would require the fluorescence intensities throughout the cross-section to be measured as a function of $r$ and $\theta$, which is not possible in our experimental setup due to the time required for a complete cross-sectional confocal scan, and the dependence of the intensity on the $z$-position.

2.3 Estimation of the effect of leakage of water through the gaps between the microgel and microchannel walls

At small pressure difference $\Delta P$ applied to the system, leakage of water may occur due to the non-conformal contact between the microgel trapped at the entrance to the constriction and microchannel walls. If the hydraulic resistance to flow through these gaps is smaller than that for the flow through the gel, the total water flow will be dominated by the leakage through the gaps, thus biasing the results. In this section, we estimate the effect of applied pressure difference, $\Delta P$, on the leakage flow and show that significant leakage occurs only for $\Delta P < E_0$.

Since the geometry of the non-conformal contact between the microgel and the wall is not known a priori, our estimation was based on the assumed gap geometry. First, we verified that a small mismatch between the spherical microgel shape and the elliptical channel cross-section cannot explain the experimentally observed pressure difference required to close the gaps. Thus we concluded that the mismatch between the channel geometry and the microgel shape should have different characteristics.
We considered the gaps between the microgel and the channel wall to be due to surface roughness (Fig. A2.1) and estimated the effective spacing $g$ of surface irregularities on microchannel wall, as well as the pressure difference, $\Delta P_c$, required to close the gaps between the microgel and the channel surface.

From Hertzian theory of an elastic contact between two spheres\[^5\], we estimate the diameter, $a$, of the contact between the spheres and applied force, $F$, as functions of strain ($h$):

$$a \approx (D_c h)^{1/2} \quad \text{and} \quad F \approx E^* (D_c h^3)^{1/2}.$$  \hspace{1cm} (A2.27)

Here, $D_c/2$ is the effective radius at the contact surface, $D_c = (1/g + 1/D_0)^{-1}$, and $E^*$ is related to the Young’s modulus, $E_0$, and Poisson’s ratio, $\sigma$, of the microgel as $E^* = 4E_0/[3(1–\sigma^2)]$, assuming rigid microchannel walls. Note that $D_c \approx g$ for $g \ll D_0$ and $E^* \approx E_0$ for $\sigma \approx 1$.

For the geometry shown in Fig. A2.1C, $L$ is the axial length of region of contacts, and $LD_0$ is proportional to the area of contact spots. Assuming the number of contacts $n \approx LD_0/g^2$, the total force imposed on the microchannel wall is $nF \approx LD_0F/g^2$. This force is supported by the pressure difference $\Delta P$. Since the radial wall pressure is proportional to $\Delta P$, the total contact force is $nF \approx LD_0F/g^2 \approx \Delta P LD_0$, and thus, the force acting on a single contact surface is $F \approx \Delta P g^2$. Substitution of the force $F$ from equation (A2.27) with $D_c \approx g$ and $E^* \approx E_0$, gives

$$\frac{\Delta P}{E_0} \approx \frac{F (E^* g^3)}{(h/g)^{3/2}}.$$  \hspace{1cm} (A2.28)

The gaps are closed when $h \approx a \approx g$. Thus, from equation (A2.28), the pressure difference $\Delta P_c$ required to close the leaking gaps is

$$\Delta P_c \approx E_0,$$  \hspace{1cm} (A2.29)

which agrees with experimental results (see Fig. 4.6A). For $\Delta P > \Delta P_c$, water flows only through the microgel without leakage.
The flow rate of water through the gaps is predicted as $Q_{\text{gap}} = \frac{\Delta P}{R_{\text{gap}}}$, where

$$R_{\text{gap}} \approx \frac{\eta}{D_0} L \left(\frac{g}{h}\right)^3 \left(\frac{g}{a}\right)^{-1}$$  \hspace{1cm} (A2.30)

is the hydraulic resistance of the gaps\[^6\]. The prefactor in square brackets is due to water leakage occurring simultaneously through $D_0/g$ gaps. The axial length of the contact region can be determined from the Hertzian theory for an elastic contact between two cylinders as\[^5\] $L \approx (F\pi F)^{1/2}$, where the force on walls is, $F \approx D_0^2 \Delta P$. Hence,

$$L \approx D_0 \left(\frac{\Delta P}{\Delta P_c}\right)^{1/2}.$$  \hspace{1cm} (A2.31)

Combining equations (A2.27-A2.31), we obtain

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**Figure A2.1 Schematics of the leaking gaps.** (A-B) Front view of the $yz$-cross-section of the microchannel and (C) contact spots on the surface of the microchannel. (A) At $\Delta P = 0$ the undeformed microgel barely touches the microchannel walls. Water can flow through the gaps of size $g$. (B) As $\Delta P$ increases, the microgel begins to conform to the microchannel surface, and (C) spots of contact areas grow on the microchannel surface.
\[
\frac{Q_{\text{gap}}}{E_0/\eta} \approx g^3 \left( \frac{\Delta P}{\Delta P_c} \right)^{1/2} \left( 1 - \left( \frac{\Delta P}{\Delta P_c} \right)^{2/3} \right)^3 \left( 1 - \left( \frac{\Delta P}{\Delta P_c} \right)^{1/3} \right),
\]

(A2.32)

which is plotted in Fig. A2.2, as blue curve.

Equation (A2.32) predicts an increase of water flux, \( Q_{\text{gap}} \sim \Delta P^{1/2} \), for a very small pressure difference, \( \Delta P \ll \Delta P_c \). This regime is followed by the shrinkage of gaps and decrease in \( Q_{\text{gap}} \), as observed in experiments (see left-most three data points in Fig. 4.6A, which correspond to the leakage-dominated regime). At \( \Delta P = \Delta P_c \), gaps close and \( Q_{\text{gap}} \) vanishes (see equation (A2.32)). At \( \Delta P > \Delta P_c \), water flows only through the microgel without leakage. In Appendix 2.4.6 “Flow resistance in the weak deformation regime”, for low \( \Delta P \), we estimated this flow through the microgel to be \( Q_{\text{gel}} = \Delta P / R_0 \), with constant hydraulic resistance \( R_0 = 1 \text{ Pa} \cdot \text{s} / \mu\text{m}^3 \) (see equation (A2.75) and red lines in Fig. 4.5B and Fig. A2.2). Hence, the total flow rate is given by \( Q = Q_{\text{gap}} + Q_{\text{gel}} \).

We estimated the size, \( g \), of gaps and the pressure, \( \Delta P_c \), required to close the gaps by fitting the theoretical prediction for \( Q(\Delta P) \) to the experimental \( Q-\Delta P \) data in the leakage dominated regime and the following linear response regime. Such a fit resulted in the best fitting parameters \( g = 0.38 \pm 0.02 \mu\text{m} \) and \( \Delta P_c / E_0 = 1.9 \pm 0.2 \), which agreed with experimental data (Fig. A2.2). We note that the reported surface roughness of PDMS surface is in the submicrometer range\(^7\).

We therefore conclude that at pressure differences \( \Delta P < E_0 \), a large number of small-roughness elements with submicrometer size can provide leakage of water comparable to the flow within the gel. The size of imperfections (on the order \( g \approx 0.1 \mu\text{m} \)), the value of \( \Delta P \) required for closing the gaps (on the order \( \Delta P_c \approx E_0 \)), and the decrease in flow rate \( Q \) with increasing \( \Delta P \) in the leakage-dominated regime (equation (A2.32) and Fig. A2.2), all agree with the experimental results. Furthermore, we estimate the hydraulic resistance of gaps as \( R_{\text{gap}} \approx 0.1 - 1 \text{ Pa} \cdot \text{s} / \mu\text{m}^3 \) from the left-most three data points in Fig. 4.6A, while the hydraulic resistance of microgel \( R_0 = 1 \text{ Pa} \cdot \text{s} / \mu\text{m}^3 \) is observed for \( \Delta P > E_0 \) in the linear response regime (equation (A2.75) and red lines in Fig. 4.5B and Fig. A2.2). Hence, by comparing the two flow resistances, we conclude that for \( \Delta P > E_0 \) the flow of water occurs primarily through the microgel. We should also note that with a cubic \((g - h)^3\) scaling, the leakage flow is highly sensitive to the height of the gaps. Thus, once the gaps are smaller than a critical height (corresponding to equal leakage and microgel flow rates), the leakage flow rapidly diminishes.
Figure A2.2 Variation of $Q$ vs. $\Delta P$ in the leakage-dominated and linear response regimes. Pressure difference dependence of $Q_{\text{gap}}$, $Q_{\text{gel}}$ and their sum $Q$ fitted to experimental data (circles) in the leakage-dominated regime and the following linear response regime with the best fitting parameters $g = 0.38 \, \mu m$ and $\Delta P/E_0 = 1.9$. Contributions to the total flow rate (black) from the flow through the leaking gaps (blue) and the microgel (red) are shown separately. Note that the leakage flow is insignificant in the higher pressure difference regime $\Delta P > E_0$ shown in Fig. 4.5.

2.4 Theoretical prediction of the behavior of microgels in confinement

2.4.1 Deformation free energy density

Consider a microgel prepared in a good solvent at the monomer number density $c_i$ that is swollen to an equilibrium concentration $c_0$ and volume $V_0$. The equilibrium swelling corresponds to the deformation factors $\lambda_0 = (c_i/c_0)^{1/3}$ in all three dimensions of the microgel. In the microchannel, the microgel is locally deformed by additional factors $\lambda_x = \lambda_{||}$ in the axial direction and $\lambda_y = \lambda_z = \lambda_{\perp}$ in the transverse directions to a new number density $c$ of monomers

$$c = c_0/(\lambda_{||} \lambda_{\perp}^2). \quad (A2.33)$$

The free energy of the microgel is the sum of elastic and osmotic components$^8$. We use the predictions of scaling theory for these two contributions to the microgel free energy. The osmotic pressure $\pi$ of the microgel in a good solvent increases proportionally to the $9/4$ power of its concentration$^{9,10}$
\[ \pi \approx (kT/b^3)(cb^3)^{9/4}, \quad (A2.34) \]

where \( b \) is the size of the monomer. The osmotic part \( f_{os} \) of the free energy per polymer chain between network junctions is proportional to the free energy density \( \sim \pi \) divided by the number of chains per unit volume \( (c/N) \), where \( N \) is the chain degree of polymerization. The osmotic contribution to the free energy per chain scales with polymer concentration \( c \) as

\[ f_{os} \approx kTN(b^3)^{5/4}. \quad (A2.35) \]

We assume that individual network chains between crosslinks deform affinely, that is, proportionally to the dimensions of the entire microgel. In this case, the dimensions of the chain in the deformed microgel is \( \bar{R}_\parallel = \lambda_0 \bar{R}_0 = \lambda_0 \lambda_0 \bar{R}_0 \) in the axial direction and \( \bar{R}_\perp = \lambda_1 \bar{R}_0 = \lambda_1 \lambda_0 \bar{R}_0 \) in the transverse direction, where \( \bar{R}_0 \) and \( \bar{R}_1 \) are the chain sizes in the fully swollen state (at \( c = c_0 \)) and in the state at which the gel was formed (at \( c = c_i \)), respectively. The elastic free energy per chain scales as

\[ f_{el} \approx kT \sum_j \left( \frac{R_j}{R_{ij}} \right)^2 \approx kT \sum_j \left( \frac{\lambda_j \lambda_0 \bar{R}_0}{R_{ij}} \right)^2, \quad (A2.36) \]

where \( \bar{R}_j \) is the amplitude of fluctuations of the chain in the deformed microgel state, and the sum is over independent \( j = \{x,y,z\} \) directions. Since the mean-square amplitude of chain fluctuations is proportional to the mean-square polymer size at semi-dilute good solvent conditions and scales as\(^{9,10}\) \( \bar{R}_j^2 \sim c^{-1/4} \), while the mean-square chain size in the preparation conditions scales as \( \bar{R}_j^2 \sim c_1^{-1/4} \), the elastic free energy per chain is

\[ f_{el} \approx kT \left( \frac{\bar{R}_j}{c_1} \right)^{1/4} \lambda_0^2 \sum_j \lambda_j^2 \approx kT \left( \frac{\bar{R}_j}{c_0} \right)^{1/4} \left( \frac{c_1}{c_0} \right)^{5/12} \sum_j \lambda_j^2. \quad (A2.37) \]

At the equilibrium swelling \( (c = c_0) \) in the absence of additional deformations, \( \sum_j \lambda_j^2 = 3 \), the total free energy of the microgel (see equations \( (A2.35) \) and \( (A2.37) \))

\[ f = f_{os} + f_{el} \approx kT \left[ N(c_0 b^3)^{5/4} + (c_i/c_0)^{5/12} \right] \quad \text{at} \ c = c_0 \quad (A2.38) \]

is minimized \( (\partial f/\partial c_0 = 0) \) at the concentration\(^{10}\)

\[ c_0 \approx \left( \frac{c_i b^3}{b^3 N^{4/5}} \right)^{1/4}, \quad (A2.39) \]
Using this relation between the concentrations at the preparation conditions and the swelling equilibrium \((c_i / c_0)^{5/12} = N(c b^3)^{5/4}\), as well as the fact that the ratio of polymer concentrations is \(c / c_0 = (\lambda_0 \lambda_1)^{-1}\) (see equation (A2.33)), one can write the total free energy per chain in the deformed state within the scaling approximation (equations (A2.35) and (A2.37)) as

\[
f \approx k T N(c_0 b^3)^{5/4} \left[ \left( \lambda_1 \lambda_2 \right)^{-5/4} + \left( \lambda_1 \lambda_2 \right)^{-1/4} \left( 2 \lambda_1^2 + \lambda_2^2 \right) \right],
\]

(A2.40)

where the sum of squares of the deformation factors in equation (A2.37) is \(\sum \lambda_i^2 = 2 \lambda_1^2 + \lambda_2^2\). Note that the free energy per unit volume of the fully swollen microgel \(f c_0 / N\) normalized by the modulus of the fully swollen gel,

\[
E_0 \approx \frac{k T}{b^3} (c_0 b^3)^{9/4},
\]

(A2.41)

is universal and depends only on the dimensionless deformation factors

\[
\frac{f}{E_0} = \frac{f c_0}{N E_0} = \frac{11}{50} \left[ \left( \lambda_1 \lambda_2 \right)^{-5/4} + \left( \lambda_1 \lambda_2 \right)^{-1/4} \left( 2 \lambda_1^2 + \lambda_2^2 \right) \right].
\]

(A2.42)

This is the reason why normalizing pressure difference \(\Delta P\) by the modulus \(E_0\) of the swollen microgels generated universal dependences in Fig. 4.7B and Fig. 4.8B. Note that we have chosen the numerical coefficient in equation (A2.42) to be 11/50, to assure that \(E_0\) is the Young’s modulus.

Below we present the scaling relations between \((i)\) the position of the microgel and the applied pressure difference, \((ii)\) the reduction in microgel volume and the degree of microgel confinement, and \((iii)\) the translocation pressure difference and the degree of confinement.

### 2.4.2 Deformation of a spherical microgel

In the steady state the size and shape of the spherical microgel under compression are determined by the condition of force balance, and below we calculate forces acting on the microgel. The walls of the microchannel impose a non-uniform deformation on the spherical microgel. Below, we describe the calculation of the deformation of a spherical microgel, neglecting the bending effects and the deformation
of the caps (Fig. A2.3). Such an approach is more applicable for the microgel confinement in a microchannel with small entrance angles $\alpha$.

The total free energy of deformation, $F$, is an integral over the whole volume of the undeformed microgel

$$F = \int_{-D_0/2}^{D_0/2} \tilde{f}[\lambda_\parallel(x_0), \lambda_\perp(x_0)]A_0(x_0) \, dx_0,$$

where $x_0$ denotes the axial coordinate in the undeformed microgel state (with the origin at the center of the microgel) and $A_0(x_0) = \frac{x_0}{4}(D_0^2 - 4x_0^2)$ is the circular cross-sectional area of the undeformed microgel at $x_0$ (Fig. A2.3).

Consider infinitesimal disk-shaped slices of the undeformed microgel. We assume that when the microgel is confined in the microchannel, each disk of diameter $\sqrt{D_0^2 - 4x_0^2}$ and thickness $dx_0$ at the axial position $x_0$ in the undeformed microgel state uniformly transforms into another disk of diameter $D(x(x_0))$ and thickness $dx$ at the axial position $x(x_0)$. Thus we neglect the bending of the disks upon deformation. The shape of the microchannel (Fig. A2.3) is the cylindrical constriction with constant diameter $D(x \geq 0) = d_c$ for the positive coordinate $x$, and the tapered truncated cone region $D(x \leq 0) = d_c - 2x \tan \alpha$, for the negative coordinate $x$. The radial and axial deformation ratios are thus

$$\lambda_\parallel(x_0) = \frac{d[x(x_0)]}{\sqrt{D_0^2 - 4x_0^2}}$$

and

$$\lambda_\parallel(x_0) = \frac{dx}{dx_0}.$$

We minimize the total free energy via standard variational method\cite{5}. In addition to the large deformation described in equation (A2.44), we define an infinitesimal deformation by $x \rightarrow x_u = x + u(x)$. This variation in the axial coordinates of the disks, $\delta x = x_u - x = u(x)$, yields the variations of the deformation factors

$$\lambda_\parallel(x_0) \rightarrow \lambda_\parallel u(x_0) = \lambda_\parallel(x_0) + \frac{d(x)}{D(x)} \lambda_\parallel(x_0) \, u(x_0) \quad \Rightarrow \quad \delta \lambda_\parallel = \frac{d(x)}{D(x)} \lambda_\parallel(x_0) \, u(x_0)$$

and

$$\lambda_\parallel(x_0) \rightarrow \lambda_\parallel u(x_0) = \lambda_\parallel(x_0) + u'(x_0) \quad \Rightarrow \quad \delta \lambda_\parallel = u'(x_0) \quad (A2.45)$$

and the variation in total free energy

$$\delta F = \int \tilde{f}[\lambda_\parallel(x_0), \lambda_\parallel u(x_0)]A_0(x_0) \, dx_0 - \int \tilde{f}[\lambda_\parallel(x_0), \lambda_\parallel(x_0)]A_0(x_0) \, dx_0.$$

(A2.46)
Substituting $\lambda_{\perp} u$ and $\lambda_{\parallel} u$ from equation (A2.45) into this $\delta F$, expanding it in series up to first order in $u$ and $u'$, and using integration by parts, we obtain

$$\delta F = \left[ \frac{\partial f(\lambda_{\perp} x_1)}{\partial \lambda_{\perp}} A_0(x_0) u(x_0) \right]_{x_0}^{x_0'} - \int \left[ \frac{d}{dx_0} \left( \frac{\partial f(\lambda_{\perp} x_1)}{\partial \lambda_{\perp}} A_0(x_0) \right) - \frac{\partial f(x)}{\partial (\lambda_{\perp} x_1)} A_0(x_0) \right] u(x_0) dx_0 . \quad (A2.47)$$

Using $\lambda_{\parallel} = dx/dx_0$, $\lambda_{\perp} = A(x)/A_0[x(x_0)]$, and defining local stress in $j$-direction

$$\sigma_{jj} = \left[ \frac{\lambda_j}{\lambda_{\parallel}^2 \lambda_{\parallel}} \right] (\partial f_{\parallel} / \partial \lambda_{\parallel}) \quad (A2.48)$$

we simplify equation (A2.47) as

$$\delta F = \left[ \sigma_{xx}(x) A(x) u(x) \right]_{x}^{x'} - \int \sigma_{xx}(x) \left[ \sigma_{xx}(x) + \frac{\partial f(x)}{\partial x} (2\sigma_{xx}(x) - \sigma_{yy}(x)) \right] A(x) u(x) dx . \quad (A2.49)$$

This extra free energy is stored in the microgel due to the conversion of the work $\delta W$

$$\delta W = - \int_{x}^{x'} P_x^{\text{int}}(x) A(x) u(x) \, dx \quad (A2.50)$$

performed on it. Here, $P_x^{\text{int}}(x)$ is the body force (force in $x$-direction per unit volume) acting on disks. Assumptions of the “undeformed caps” and “no bending of disks” imply zero stress at the back and front boundaries of the deformed part of the microgel

$$\sigma_{xx}(x_b') = \sigma_{xx}(x_f') = 0 . \quad (A2.51)$$

Note that $\sigma_{xx}(x_b') = 0$ requires $\lambda_{\parallel}(x_b') = \lambda_{\parallel}(x_f') = 1$ at the front and back boundaries of the deformed section of the microgel. Inside the deformed part of the microgel $P_x^{\text{int}}(x) = -P'(x)$ results in

$$\sigma_{xx}(x) + \frac{\partial f(x)}{\partial (\lambda_{\perp} x_1)} (2\sigma_{xx}(x) - \sigma_{yy}(x)) + P'(x) = 0 , \quad (A2.52)$$

where $P'(x)$ is the water pressure gradient. For a steady state, these conditions correspond to the force balance at the back and front boundaries of the deformed part of the microgel (equation (A2.51)) and inside the deformed section of the microgel (equation (A2.52)), respectively.
Axial coordinates $x_0$ and $x$ are used for the undeformed and deformed states of the microgel; $x_0 = 0$ corresponds to the center of the unperturbed microgel and $x = 0$ corresponds to the entrance of the constriction. We assume that a disk-shaped thin slice in undeformed state at position $x_0$ with respect to its center deforms homogeneously without bending into a disk-shaped thin slice at $x(x_0)$. Front and back microgel tip positions are $x_f$ and $x_b$, respectively, while the front and back boundary positions of the deformed gel are $x'_f$ and $x'_b$ (axial boundaries of microgel portion in contact with the microchannel walls). We neglect the deformation of the back and front caps (dark shaded regions) assuming $\lambda_{\perp} = \lambda_{\parallel} = 1$ inside the caps ($x_b \leq x \leq x'_b$ and $x'_f \leq x \leq x_f$). With these boundary conditions, we numerically solve the differential force balance equations (A2.55) and (A2.56).

2.4.3 Pressure gradient along the microgel

In order to relate the water pressure gradient $P'(x) = dP/dx$ to the deformation along the microgel, we modeled each elementary vertical slice of a microgel (a disk of diameter $D(x)$ and thickness $dx$) at the axial position $x$ by a densely packed set of parallel "pipes" with a diameter $\xi(x)$ (the correlation length), which scales with polymer concentration as $\xi = \xi_0 \left( c/c_0 \right)^{-3/4}$; thus, $\xi = \xi_0 \left( \lambda_{\perp}^2 \lambda_{\parallel} \right)^{3/4}$ (see equation (A2.33)). The pressure difference $dP$ across the pipe is $dP = q \, dR_{\xi}$, where $dR_{\xi}$ is the hydraulic resistance of each pipe and $q$ is the volumetric flow rate of water through the pipe (e.g., in units of $\mu$m$^3$/s). Each disk of diameter $D(x)$ and length $dx$ accommodates $m \approx \left( D/\xi \right)^2$ pipes, and the total resistivity of a disk containing $m$-pipes acting in parallel is $dR = dR_{\xi}/m$. The flow rate through the disk is $Q = mq$. 

Figure A2.3 Schematics of microgel confinement. Axial coordinates $x_0$ and $x$ are used for the undeformed and deformed states of the microgel; $x_0 = 0$ corresponds to the center of the unperturbed microgel and $x = 0$ corresponds to the entrance of the constriction. We assume that a disk-shaped thin slice in undeformed state at position $x_0$ with respect to its center deforms homogeneously without bending into a disk-shaped thin slice at $x(x_0)$. Front and back microgel tip positions are $x_f$ and $x_b$, respectively, while the front and back boundary positions of the deformed gel are $x'_f$ and $x'_b$ (axial boundaries of microgel portion in contact with the microchannel walls). We neglect the deformation of the back and front caps (dark shaded regions) assuming $\lambda_{\perp} = \lambda_{\parallel} = 1$ inside the caps ($x_b \leq x \leq x'_b$ and $x'_f \leq x \leq x_f$). With these boundary conditions, we numerically solve the differential force balance equations (A2.55) and (A2.56).
The flow of liquid inside the pipes is assumed to be laminar and described by the Poiseuille law, which gives the resistance of a pipe \(dR = (128 \eta \, dx)/(\pi \, \xi^4)\), where \(\eta\) is the viscosity of the liquid\(^6\). Thus the resistivity of a disk at position \(x\) is

\[
dR = (128 \eta \, dx)/[\pi D^2(x) \xi^2(x)] \tag{A2.53}
\]

and the pressure gradient at position \(x\) is \(dP/dx = (128 \eta \, Q)/(\pi \, D^2(x) \xi^2(x))\). Note that Eq. (A2.53) can be treated as Darcy’s law for the flow through a porous medium with non-uniform permeability along the microgel as \(\kappa = \xi^2/32\), since Darcy’s law predicts\(^6\) \(dR = (4\eta dx)/(\pi D^3 \kappa)\). By substituting the correlation length \(\xi = \xi_0 (\lambda_{\perp}^2 \lambda_{||})^{3/4}\) for good solvent conditions, we obtain

\[
dP/dx = [(128 \eta \, Q)/(\pi \, \xi_0^2)] D^{-2}(x) [\lambda_{\perp}^2(x) \lambda_{||}(x)]^{-3/2}, \tag{A2.54}
\]

which must be balanced by the body force inside the deformed microgel, as was given in equation (A2.52). By using the geometry of the setup and the free energy density of the microgel (given in equation (A2.42)) in the calculation of the stress components \(\sigma_{ij}\), we obtain

\[
\frac{\lambda_{\perp}'(x)}{\lambda_{\perp}(x)} = \frac{-2}{D(x)} \left[ \frac{d'(x)[45 - 30 \lambda_{\perp}^2(x) \lambda_{||}(x) - 3 \lambda_{\perp}^2(x) \lambda_{||}^2(x)] + G(x)[45 + 2 \lambda_{\perp}^2(x) \lambda_{||}(x) - 3 \lambda_{\perp}^2(x) \lambda_{||}^2(x)] - \frac{10}{D(x)} \lambda_{\perp}^2(x) \lambda_{||}(x)]^{3/4}}{45 + 10 \lambda_{\perp}^2(x) \lambda_{||}(x) + 21 \lambda_{\perp}^2(x) \lambda_{||}^2(x)} \right],
\tag{A2.55}
\]

where \(\lambda_{\perp}' = d\lambda_{\perp}/dx\), and we have used an abbreviation for the characteristic flow rate (in units of length), \(\ell_0 = (51200 \eta \, Q)/(11\pi \, \xi_0^2 E_0)\). Note that \(\ell_0 \sim Q\) appears in \(\lambda_{\perp}'/\lambda_{\perp}\) in the above equation since flow deforms the microgel in the axial direction. In addition to equation (A2.55), the definition of \(\lambda_{\perp}\) given in equation (A2.44), leads to

\[
\frac{\lambda_{\perp}'(x)}{\lambda_{\perp}(x)} = \frac{d'(x) + G(x)}{D(x)}, \tag{A2.56}
\]

where \(\lambda_{\perp}' = d\lambda_{\perp}/dx\). In equations (A2.55) and (A2.56), we used a function

\[
G(x) = \mp \frac{2 \lambda_{\perp}^2(x)}{\lambda_{||}(x)} \sqrt{\left[ \frac{D_0}{D(x)} \right]^2 - \frac{1}{\lambda_{||}^2(x)}}, \tag{A2.57}
\]
Here, the positive and negative signs are for the two sides of the unperturbed microgel, $x_0 < 0$ and $x_0 > 0$ respectively, as the unperturbed diameter of a disk increases or decreases with $x_0$, depending on which side of the gel it is.

### 2.4.4 Results of numerical simulations

The coupled differential equations (A2.55) and (A2.56), corresponding to the force balance inside the microgel and the shape of the microgel imposed by the confinement are numerically integrated via 4th order Runge-Kutta algorithm\(^3\) for the geometrical parameters relevant to the geometry of the microchannel. The assumption of the undeformed microgel caps corresponds to the boundary conditions of $\lambda_{\perp}(x') = \lambda_{\parallel}(x') = 1$ at the back and front boundaries of the microgel portion in contact with the microchannel walls. Note that the definition of $\lambda_0$ in equation (A2.44) implies $dx_0 = dx/\lambda_0$, which can be used to transform the integrals over $x_0$ in the undeformed state into integrals over $x$ in the deformed state. Thus we obtained the deformation ratios $\lambda_{\perp}(x|x')$, $\lambda_{\parallel}(x|x')$ and the normalized axial stress $\sigma_{xx}(x|x')/E_0$ inside the microgel at the axial position $x$ for a particular front boundary position $x'$. The flow rate $Q$ was adjusted by a bisection algorithm to obtain the undeformed back boundary.

In order to determine the position $x_c$, where the function $G(x)$ (introduced in equations (A2.55) and (A2.56)) changes its sign, we also performed an axial length integration

$$
S(x) = \int_{x_0}^{x_0'} d\tilde{x}_0 = \int_x^{x'} \lambda_{\parallel}^{-1}(\tilde{x}) d\tilde{x}.
$$

(A2.58)

From the geometry defined in Fig. A2.3, the center of the undeformed microgel ($x_0 = 0$) corresponds to

$$
S(x_c) = \frac{1}{2} \sqrt{D_0^2 - D_I^2}.
$$

In Fig. A2.4, we illustrate the numerical results of our theoretical approach described above. Figs. A2.4A and B show the numerical results for profiles of deformation ratios ($\lambda_{\perp}$, $\lambda_0$) and normalized concentration ($c/c_0$) along the deformed microgels at different positions inside the microchannel. At translocation
instability point (at \( \Delta P = \Delta P_{\text{max}} \), grey lines), the largest microgel compression is at the constriction entrance \( (x = 0) \). Based on Fig. A2.4B, the respective increase in polymer concentration is in the range \( 1 \leq c/c_0 \leq 3.3 \). Recalling that \( \kappa/\kappa_0 = (\xi/\xi_0)^2 = (c/c_0)^{-3/2} \), we conclude that the ratio of permeabilities \( \kappa/\kappa_0 \) varies in the range \( 1 \geq \kappa/\kappa_0 \geq 0.2 \). Here \( \kappa_0 \) is the permeability of the undeformed microgel, which was estimated to be approximately 7 nm² from Eq. (A2.75). For the highest increase in polymer concentration of \( c/c_0 \approx 3.3 \), the largest reduction in permeability by the factor of 5 (or the smallest ratio \( \kappa/\kappa_0 \approx 0.2 \)) was obtained. In Fig. A2.4C, we plot the local volume change factor \( (\lambda_{\perp}, \lambda_{||}) \) as a function of local radial deformation factor \( (\lambda_{\perp}) \) using the data in Fig. A2.4A. The relation \( \lambda_{\perp}^2 \lambda_{||} \sim \lambda_{\perp}^{4/3} \) (obtained for no external axial stress from equation (A2.63), dashed line) is in good agreement with the numerical data. In Fig. A2.4D, we plot the calculated flow rate as a function of pressure difference and compare the numerical data with scaling predictions. Finally, Fig. A2.4E shows the numerical result for the normalized front tip position of the microgel in the constriction, \( x_f/d_c > 0 \), as a function of normalized pressure difference, \( \Delta P/E_0 \), compared to experimental data. Fitting a function in the power-law form, \( x_f/d_c \approx (\Delta P/E_0)^a \), to the numerical data leads to \( a = 2.3 \pm 0.1 \) for a broad range of pressure differences. The universal scaling relation

\[
\frac{x_f}{d_c} \approx (\frac{\Delta P}{E_0})^{2.3} \quad \text{for} \quad x_f > 0,
\]  

(A2.59)
is also in agreement with the experimental results for the strongly constricted microgels. In the next section, we discuss scaling relations for volume reduction, \( V/V_0 \), and rescaled translocation pressure difference \( \Delta P_{\text{max}}/E_0 \).
Figure A2.4 Numerical results for the deformation ratio profiles, normalized concentration profiles, volume change factor, normalized flow rate, and normalized front tip position of the microgel in the constriction. Geometrical parameters are $\alpha = 30^\circ$, $d_c = 38 \mu m$, $D_0 = 103 \mu m$ in (A-D) and $\alpha = 15^\circ$, $d_c = 42 \mu m$, $D_0 = 94 \mu m$ in (E). Qualitatively similar results are obtained for different sets of geometrical parameters. (A) Deformation ratios (ratios of deformed to undeformed lengths) in axial $\lambda_||$ (upper solid curves) and radial $\lambda_\perp$ (lower dotted curves) directions as functions of axial coordinate $x$ along the deformed microgel normalized by the constriction diameter $d_c$. Different colors correspond to different microgel locations: microgel completely localized in the tapered region ($x'_f < 0$, red lines); microgel at the point of entry into the constriction ($x'_f = 0$, green lines); partially constricted before translocation ($x'_f > 0$, $\Delta P < \Delta P_{\text{max}}$, blue lines), and microgel at the translocation instability point ($\Delta P = \Delta P_{\text{max}}$, grey lines). The
vertical lines at \( x = 0 \) mark the constriction entrance in (A) and (B). (B) Concentration profile along the confined microgels. Colors correspond to the same microgel locations along tapered and constriction zones as in (A). Note that for microgel partially in constriction (blue and grey lines) the maximum concentration is near the boundary between tapered zone and constriction. (C) Dependence of the relative change in volume \( \lambda_{\perp}^2 \lambda_{||} \) on the radial deformation ratio \( \lambda_{\perp} \). Logarithmic axes. Colors correspond to the same microgel locations along tapered and constriction zones as in (A). Black dashed line representing the asymptotic dependence \( \lambda_{\perp}^2 \lambda_{||} \sim \lambda_{\perp}^{4/3} \) (obtained from equation (A2.63)) predicted for the case of no external axial stress is in reasonable agreement with the numerical solutions in the presence of external axial stress due to flow through the microgel (colored lines). (D) Dependence of the flux \( Q \) (normalized by \( E_0/R_0 \) see equation (A2.73)) through the microgel on the pressure difference \( \Delta P \) across it (normalized by \( E_0 \)). Solid black curve shows numerical calculation described in the Appendix 2.4.4, including flow through the caps. Red line shows asymptotic linear dependence \( Q \sim \Delta P \) for low pressure differences. Blue line shows asymptotic scaling \( Q \sim \Delta P^{-2.0} \) for high pressure differences. Colored circles correspond to the same microgel locations as in (A)-(C). (E) Normalized front tip position of the microgel in the constriction \( x_{f}/d_{c} \) as a function of normalized pressure difference \( \Delta P/E_0 \). Black curve is the result of the numerical calculations for \( \alpha = 15^\circ, d_{c} = 42 \mu m, D_0 = 94 \mu m \). Markers show the experimental results (see Fig. 4.7B) for these geometrical parameters. The red line with a slope 2.3 (see equation (A2.59)) is in good agreement with numerical and experimental results over a wide range of \( \Delta P/E_0 \) for the strongly constricted microgel. This scaling relation (equations (4.2) and (4.6)) is used in the theoretical analysis of experiments (equations (4.3 – 4.5) in Chapter 4).

2.4.5 Scaling predictions for a deformed microgel

In the sections from Appendix 2.4.2 to 2.4.4 above, we considered spherical microgels. Here, in order to obtain universal scaling predictions, we consider a microgel with a cylindrical undeformed shape and an equilibrium swollen diameter \( D_0 \) with its symmetry axis oriented in \( x \)-direction.

2.4.5.1 Volume reduction of a microgel in the constriction

If a microgel is completely inside the constriction at zero pressure difference \( \Delta P = 0 \) and no fluid flows through it \( (Q = 0) \), the microgel is unconstrained in the axial direction. The radial size of the microgel is equal to the diameter \( d_{c} \) of the constriction. Thus the radial deformation factor is constant \( \lambda_{\perp} = d_{c}/D_0 \). The longitudinal deformation factor \( \lambda_{||} \) of the microgel can be obtained by the minimization of the free energy per chain (equation (A2.40)) or the free energy per unit volume of the fully swollen microgel (equation (A2.42)) with respect to \( \lambda_{||} \) at constant \( \lambda_{\perp} = d_{c}/D_0 \). This minimization, \( \partial f/\partial \lambda_{||} = 0 \), corresponds to the balance of elastic and osmotic stresses in the unconstrained axial direction

\[
\frac{\partial f}{\partial \lambda_{||}} \approx \frac{11}{50} E_0 \left( -\frac{5}{4} \lambda_{||}^{-9/4} \lambda_{\perp}^{-5/2} - \frac{1}{2} \lambda_{||}^{-5/4} \lambda_{\perp}^{3/2} + \frac{7}{4} \lambda_{||}^{3/4} \lambda_{\perp}^{-1/2} \right)
\]
\[ \lambda_{||} = \frac{5}{7} (D_0/d_0)^{2/3} \left[ \left( \frac{1}{2} + v \right)^{1/3} + \left( \frac{1}{2} - v \right)^{1/3} \right], \quad (A2.61) \]

where

\[ v = \left[ \frac{1}{4} - \left( \frac{8}{4725} \right) (D_0/d_0)^{-10} \right]^{1/2}. \quad (A2.62) \]

Note that for large radial compressions \( \lambda_\perp \ll 1 \), the parameter \( v \approx \frac{1}{2} \) and

\[ \lambda_{||} \sim \lambda_\perp^{-2/3} \quad \text{for} \quad \lambda_\perp \ll 1. \quad (A2.63) \]

The relative change of volume upon microgel confinement in the constriction is

\[ V/V_0 = \lambda_\perp^2 \lambda_{||} = \left( \frac{5}{7} \right)^{1/3} (D_0/d_0)^{-4/3} \left[ \left( \frac{1}{2} + v \right)^{1/3} + \left( \frac{1}{2} - v \right)^{1/3} \right], \quad (A2.64) \]

which is equation (4.8) in Chapter 4.

The above equation (A2.64) (equation (4.8) in Chapter 4) is obtained for a cylindrical shape of the undeformed microgel. The relations show a universal power-law dependence with an exponent \(-4/3\) for geometry confinement, and are represented by the black curves in Figs. 4.9A and B in Chapter 4. The experimental and theoretical results were in excellent agreement without any fitting parameters.

### 2.4.5.2 Dependence of the translocation pressure on the degree of microgel confinement

The dependence of \( \Delta P_{\text{max}} \) on \( D_0/d_0 \) can be obtained by integrating equation (A2.52) along the microgel:

\[ \Delta P = - \int_{x_b}^{x_f} dP = \int_{x_b}^{x_f} d\sigma_{xx} + \int_{x_b}^{x_f} \frac{dx}{\lambda_4} \left[ \frac{d}{dx} \frac{\sigma_{xx}}{\lambda_{||}} \right], \quad (A2.65) \]

The first integral vanishes due to vanishing surface forces (equation (A2.51)), and using \( \int_{x_b}^{x_f} \frac{dx}{\lambda_{||}} \approx D_0 \), we estimate the second integral in equation (A2.65) as...
Here, the right-hand-side is taken at a certain point \( x = x_m = -\gamma(\alpha) d_c \), near the maximum of the expression in square brackets in equation (A2.65) inside the integration interval \( (x'_b \leq x \leq x'_e) \) at the translocation instability point \( \Delta P = \Delta P_{\text{max}} \). For strong deformation \( (\lambda_{\parallel} \approx \lambda_{\perp}^{-2/3} \gg \lambda_{\perp}) \), from equations (A2.42) and (A2.48), we have \( 2\sigma_{xx} - \sigma_{rr} \approx E_0 \lambda_{\perp}^{-3} \), and substituting it into equation (A2.66) along with the slope of the tapered zone, \( i.e., \frac{dD}{dx} = -2 \tan\alpha \), we write

\[
\Delta P_{\text{max}} \approx \left[ -D_0 \frac{\lambda_{\parallel} dD}{dx} (2\sigma_{xx} - \sigma_{rr}) \right]_{x_m}.
\]  

(A2.66)

Here, making use of the cylindrical undeformed gel approximation, \( i.e., \lambda_{\perp} \approx D/D_0 \), we obtain

\[
\frac{\Delta P_{\text{max}}}{E_0} \approx \tan\alpha \left[ \frac{D_0 \lambda_{\perp}^{-11/3}}{dD/dx} \right]_{x_m}.
\]

(A2.67)

The radial deformation ratio at \( x = x_m \) is \( \lambda_{\perp}(x_m) = D(x_m)/D_0 = (d_c - 2 \tan\alpha x_m)/D_0 = (1 + 2 \gamma(\alpha) \tan\alpha) (d_c/D_0) \). Therefore, we have

\[
\frac{\Delta P_{\text{max}}}{E_0} = K(\alpha) \left( \frac{D_0}{d_c} \right)^{14/3},
\]

(A2.69)

where we defined the angle-dependent prefactor

\[
K(\alpha) \approx \tan\alpha \left( 1 + 2 \gamma(\alpha) \tan\alpha \right)^{14/3}.
\]

(A2.70)

The size dependence of translocation pressure difference, \( i.e., \) the power-law \( \Delta P_{\text{max}} \approx (D_0/d_c)^{14/3} \), is universal with an exponent 14/3, independent of the shape of the tapering zone. However, the prefactor (amplitude) of this power-law, \( K(\alpha) \), is not universal and depends on the geometry of the microchannel and the microgel. To this end, we consider simulation results to obtain the amplitude \( K(\alpha) \) and compare them with experimental data.

In Fig. A2.5A, we plot the simulation results for rescaled translocation pressure difference, \( \Delta P_{\text{max}}/E_0 \), as a function of degree of confinement, \( D_0/d_c \), for different tapering angles, \( \alpha \). We observe (i) persistent power-law behavior \( \Delta P_{\text{max}} \approx (D_0/d_c)^{14/3} \), and (ii) saturation of the angle-dependent amplitude for large angles. This
saturation is evident in Fig. A2.5B, in which we plot the amplitude, $K(\alpha)$. We should again note that, in these numerical simulations, we neglected the bending effects, which are more pronounced at large tapering angles and large degrees of confinements. In order to capture the saturation of $K(\alpha)$ to a finite value for large angles and to reproduce $K(\alpha) \approx \tan \alpha$ for small angles, we choose $\gamma(\alpha) \approx (\tan \alpha)^{-11/14}$, which yields

$$K(\alpha) \approx [k + (\tan \alpha)^{-3/14}]^{-14/3}, \quad (A2.71)$$

where $k$ is a numerical constant that can be obtained by the fit to experimental data.

In Fig. A2.6A, we verify the above approximation by plotting the experimental data (see Fig. 4.8B) in the form $(\Delta P_{\text{max}}/E_0)^{-3/14} (D_0/d_c)$ as a function of $(\tan \alpha)^{-3/14}$. Fit of the function $[K(\alpha)]^{-3/14}$ by a linear dependence (predicted as in equation (A2.71)) yields a best fitting parameter $k = 0.72 \pm 0.02$. In Fig. A2.6B, we present the log-log plot of the experimental $\Delta P_{\text{max}}/E_0$ data rescaled with $K(\alpha)$ for $k = 0.72$, and observe that data for all angles collapse on a single line with a slope 14/3 as predicted by equation (A2.69).

Figure A2.5 Translocation pressure difference and angle-dependent amplitude $K(\alpha)$. (A) Simulation results for the rescaled translocation pressure difference, $\Delta P_{\text{max}}/E_0$, as a function of degree of confinement, $D_0/d_c$, for different tapering angles, $\alpha = 15^\circ$ (blue), $45^\circ$ (red), $60^\circ$ (green), and $90^\circ$ (black). The power-law dependence, $\Delta P_{\text{max}} \approx (D_0/d_c)^{14/3}$ (shown with the dotted line), is persistent for all angles. (B) Angle-dependent amplitude of this power-law, $K(\alpha)$, at degree of confinement $D_0/d_c = 2$ for the angles shown in (A). Same colors are used to indicate different angles as in (A). The amplitude $K(\alpha)$ saturates to a constant for large angles.
Figure A2.6 Master curves for translocation pressure difference. (A) Experimental data shown in Fig. 4.8B plotted in the form \((\Delta P_{\text{max}}/E_0)^{3/14}(D_0/d_c)\) as a function of \((\tan\alpha)^{3/14}\). Data in this form is fit to the predicted function, \([K(\alpha)]^{3/14} = k + (\tan\alpha)^{3/14}\) (see equation (A2.71)). The resulting best fitting parameter is \(k = 0.72\) as shown with the black curve. (B) Experimental \(\Delta P_{\text{max}}/E_0\) data rescaled with \(K(\alpha)\) for \(k = 0.72\), plotted versus \(D_0/d_c\) (in log-log axes). Data for all angles collapse on a single line with a slope 14/3 (shown in black). Both in (A) and (B), different colors indicate different tapering angles: \(\alpha = 15^\circ\) (blue), 45\(^{\circ}\) (red) and 60\(^{\circ}\) (green), as in Fig. 4.8B and Fig. A2.5B.

2.4.6 Flow resistance in the weak deformation regime

Consider a microgel weakly deformed in the tapered zone under a small pressure difference, \(\Delta P \ll E_0\), where \(E_0\) is the modulus of the microgel. To calculate the flow resistance of the weakly deformed spherical gel, we integrate the reciprocal resistance of “pipes” of diameter \(\xi_0\), over the concentric rings (see Fig. A2.7) of length \(L(r) = 2 [(D_0/2)^2 - r^2]^{1/2}\) and cross-sectional area \(dA = 2\pi r \, dr\) (in the range \(0 < r < D_0/2 - \delta\)), i.e.,

\[
\frac{1}{R} = \frac{\pi}{128} \frac{\xi_0^2}{\eta} \int_{L(r)} \frac{dA}{dA} = \frac{\pi}{128} \frac{\xi_0^2}{\eta} \int_0^{D_0/2 - \delta} \frac{2\pi r \, dr}{2\sqrt{(D_0/2)^2 - r^2}}
\]

\[
= \frac{\pi^2 \xi_0^2}{256} \frac{D_0}{\eta} - 2\sqrt{(D_0 - \delta)} = \left(\frac{\pi}{16}\right)^2 \frac{\xi_0^2}{\eta} \left[D_0 - L\left(\frac{D_0}{2} - \delta\right)\right], \quad (A2.72)
\]

where \(L(D_0/2 - \delta)\) is the length of the contact zone \(L(D_0/2 - \delta) = 2\sqrt{\delta(D_0 - \delta)}\). The resistance \(R\) calculated in equation (A2.72) is inversely proportional to the thickness of the caps \(D_0 - L(D_0/2 - \delta)\). At small deformations \((\delta \ll D_0)\) the flow resistance is equal to that of the unperturbed microgel

\[
R = R_0 = \frac{(16/\pi)^2 \eta(\xi_0^2 D_0)}{} \quad \text{for} \quad \delta \ll D_0 \quad (A2.73)
\]
and is therefore constant independent of compression $\delta$ and pressure difference $\Delta P$. This implies that most of the fluid flows through the whole microgel rather than through the small volume in the vicinity of the compressed microgel section of size $L(D_0/2 - \delta)$. The high resistance of longer pores (with length $\sim D_0 \gg L(D_0/2 - \delta)$) that pass through the central section of the microgel is compensated by the smaller number of such pipes. Thus we conclude that at small pressure difference, $\Delta P \ll E_0$, the flux $Q$ of water through the microgel is linearly proportional to the pressure difference

$$Q \approx \Delta P/R_0,$$  \hspace{1cm} (A2.74)

in good agreement with numerical calculations (red line with unit slope in Fig. A2.4D) and experiments (red line with unit slope in Fig. 4.5B).

Balance of elastic and osmotic stresses at undeformed equilibrium state leads to\[10\] $E_0 \approx 2kT/\xi_0^3$. For the Young’s modulus $E_0 = 2570$ Pa of the microgel used in the flow experiments, and $kT \approx 4.11 \cdot 10^{-3}$ Pa·μm³ at room temperature, we estimate the pore size in undeformed microgel to be $\xi_0 \approx 15$ nm. Substituting this value into the equation (A2.73) for the resistance $R_0$ of the undeformed gel of diameter $D_0 \approx 100$ μm with viscosity $\eta \approx 0.9 \cdot 10^{-3}$ Pa·s for water at room temperature, we obtain $R_0 \approx 1$ Pa·s/μm³. This estimate is in excellent agreement with the experimental results – the best linear fit to the data (red line in Fig. 4.5B) gives

$$R_0 = 1 \text{ Pa} \cdot \text{s/μm}^3.$$  \hspace{1cm} (A2.75)
Figure A2.7 Weak indentation by $\delta$ of a spherical microgel of diameter $D_0$ inside the tapered zone with the small angle $\alpha \ll 1$. The radius of the tapered microchannel in the contact zone is $D_0/2-\delta$. The axial length of the contact between microgel and microchannel is $L(D_0/2-\delta)=2\sqrt{\delta(D_0-\delta)}$. The resistance $R$ is obtained by integration over cylindrical rings of radius $r$, thickness $dr$ and length $L(r)$. 
References


